# CHEMICAL CHARACTERIZATION OF SWAMP PEAT HUMIC SUBSTANCES

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CHEMICAL CHARACTERIZATION OF

SWAMP PEAT HUMIC SUBSTANCES

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"Don't ask me, I don't give a damn." Country Joe McDonald

"Don't ask me nothin' about nothin', I just might tell you the truth." Bob Dylan

"Because it's finite." Jack Harich

"Nice guys finish last, but have a good time getting there." Derrold

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## SUMMARY

The source and chemical nature of the natural organic geopolymers occuring in rivers is of decided importance in understanding the river system and our potential effect upon it. To gain some insight into these compounds it was decided to extract organic material from the Okefenokee Swamp Complex, a somewhat defined source area rich in these molecules. Twelve humic acids, one fulvic acid, one river water organic matter and twelve water extractable samples are examined. This material is then characterized using the methods commonly applied to these substances.

The chemical activity is evaluated using standard analyses for total acidity, carboxyl acidity, phenolic acidity, carbonyl content and amino acid content. Some insight into the physical structure of the macromolecules is gained through UV/visible spectrophotometer and especially through detailed study of the samples and various derivitives. Emphasis is placed on the water extractable material as it is most likely to appear in the river.

The aqueous extracts are found to have functional group contents similar to the literature values found for fulvic acids and river water organic matter. There is, however, no discernable relationship between the parent biota and any functional group content. Spectroscopic data is interpreted as suggesting that the polymers are structurally quite similar to fulvic acids but are of a lower molecular weight. It is also

found that the aqueous extracts are not identical to the material found in solution in the river. This seems to indicate that the polymers undergo chemical reations and/or fractionation after they are initially mobilized by dissolution in ground water.

It is noteworthy that two of the most commonly applied functional group analysis procedures, total acidity by reation with barium hydroxide and carboxyl acidity by reaction with calcium acetate are both suspected of giving incorrect results.

# CHAPTER I

## INTRODUCTION

In recent years a growing concern over water resources and our effect on them has led to increasing research on the chemistry of natural waters. A generally rather minor component, the natural organic geopolymers, have been found to have a large effect, or potential effect, on river chemistry. The chemical heterogeneity of these geopolymers enable them to react chemically in a variety of ways; e.g. chelation, exchange and buffering (Beck et al. 1974). Potential interaction of this organic component with heavy metals (Dunn 1974, Andren 1975, Reuter and Perdue 1977) and herbicides (Khan 1973) indicates a definite need for a better understanding of the chemistry of the organic material dissolved in river water.

In the past quantitative studies of the dissolved organic components of river water have been scarce and incomplete. Packham (1974) studied river organic matter (ROM) from the Thames River and concluded that it largely resembled fulvic acid. Christman and Chassemi (1966) conclude with a description of ROM which resembles that of a humic or fulvic acid. In our own laboratory considerable effort has been directed toward more quantitative studies (Reuter and Perdue 1977, Martin 1973, Dunn 1974). This data gives a picture of ROM as similar to, but not exactly, fulvic acid. Fulvic acid is an operationally defined fraction of organic matter. It is the material left in

solution upon acidification of a basic organic matter extract. The material which flocculates out of the solution is the humic acid.

Furthermore, the exact origin of these molecules is uncertain. Certainly the similarity of river organic matter to soil organic matter (humic and fulvic acids) suggests that river organic matter originates in the soil. There are however sufficient differences to show that river organic matter is not simply dissolved soil humic material.

It was thus decided to study the organic material mobilized by water from a soil system and compare it to the river organic matter found in the rivers draining the area. Data already existed in our laboratory for Suwannee River organic matter and it was decided to study the peat soil of the Okeefenokee Swamp complex which is the source for the river. The peat samples were selected from well defined but botanically widely varied parent biota by following the petrographic work of Cohen (1974) in the same area. From these could be extracted various humic components which could be chemically well defined. These data are directly comparable to a large body of data already existing for soil humic and fulvic acids as well as for river organic matter (Beck et al. 1974, Martin 1973).

# CHAPTER II

## PROCEDURES

Samples for this study were collected in the Okeefenokee Swamp of southeastern Georgia, using the petrographic study of Cohen (1974) as a guide (Fig. 1). Every attempt was made to obtain samples with a consistent and defined biological origin. Regions within the swamp were selected on the basis of Cohen's core analyses and to give as much coverage to the swamp as possible. Once within the selected region, identification of the biota was made and samples were taken as far from the boat trails as practicable. Samples were taken from 6-12 inches under the surface of the peat which itself was underwater. Following is a stricter definition of the samples.

### Taxodium (Cypress)

These samples come from the forested regions of the Okeefenokee Swamp. The biota are primarily cypress, bays, gums and shrubs (Cohen).

Sample #7 Taken from the location designated #7 by Cohen. Water was standing about 2 1/2 ft. Peat is noticeable reddish. Numerous roots at sample depth (6-12 inches).

Sample #11 Taken 1/4 mile into taxodium forest from Cohen's sample #6. Water standing 3 1/2 ft. Sample taken from 3-6 inches into the peat; again the color is noticeably reddish and full of living roots.

When taking these two samples it is noticed that no "marsh gas" is released.

Nymphaea (Prairie)

Sample #9 Taken from same region as Cohens core #9. Water is 2 1/2-3 ft. deep and all biota is clearly submerged. Considerable gas is released upon digging. Peat is noticeably free from roots and is easily scooped out. Brown in color.

Sample C Taken very near sample #9 from a "floating battery" associated with a main root from a water lily.

Sample #8 Taken just North of canoe trail to Floyds Island. Waterlily and Maidercane are dominant biota. Water standing 3 1/2 ft. deep, sample taken 4-8 inches into the bottom.

Sample #14 Taken 1 mile North of Monkey Island. Biota noted was Yellow Eyed Grass (Xyris sp), Nymphaea odorata, Nymphoides aquaticum (Floating Heart) and Golden Club (Orontium Aquaticum). Moderate amount of gas evolved on taking sample.

Sample #15 Taken near Cooter Lake. Basically the same as #14 - Chesser Prairie Sample.

Pine Forest

Sample B Taken near Lee cemetery on Billy's Island. Pine forest is dominant biota; ground cover is pine straw on sand. Color of soil is dark gray-black on surface tapering to light brown 1 1/2 ft. down. Sand is later seen to be white after NaOH extraction.

Flocculate ROM

Sample H Taken at Crooked River State Park from shore of Crooked River estuary about 8 miles from open ocean. Chunks of flocculate ROM lie all along the shore. After extraction with $H_2O$ and then NaOH solution

only quartz sand was left behind.

## Emergent Aquatics

These samples come from the island-fringe areas. Biota are chiefly Woodwardia Virginica (chain fern), Panicum hemitomon (maidencane) and Pitcher plant.

Sample #12 Taken 1/4 mile east of Gannett Lake near a small tree island. Water standing 1-1/2 ft. deep. Large number of tenatious roots.

Note that here water lilies are white (Nym odorata), while on the West side of the swamp they are yellow.

Sample #13 Taken from edge of Monkey Island in the Grand Prairie. Most roots are in upper 3-5 inches of peat. Woodwardia and Maidencane are prevalent. Some gas evolved on taking sample.

## R O M

RS 36

This sample is dissolved river water organic material. It is taken from down stream in the Satilla River. This sample has been extensively analyzed by Martin (1973).

## Sample Extraction

The samples are first soaked in 20 liter carboys with distilled $H_2O$. This is done twice on each sample. The water is filtered to remove the peat and then centrifuged to remove any small particulate matter. The centrifuging is done using a Sorvall SS-3 high speed centrifuge operated in a flow through mode. Operating parameters are 15,000 rpm and a flow rate of 3 liter per hour. This should remove all

particulate matter greater than .1 micron. Table 1 shows the absorbance of the aqueous solutions after this treatment. The peat is then dried in the air. The filtered extract is concentrated approximately 15 fold and then freeze-dried. This yields a light, fluffy powder light brown to tan in color. These samples are termed aqueous (Aq) in this paper. After total acidity analysis the samples are desalted by dissolving the sample in 1 liter distilled $H_2O$ and passing through a 5-7 fold excess of Bio Rad AG 50W X8 ion exchange resin. The solution is then freeze dried.

Next 100 gm of dried peat is put into a 20 liter carboy extracted with .05M NaOH. Samples are left to stand overnight after careful $N_2$ flushing of the solution before and after addition to the peat. The first 2 extractions are acidified, frozen, thawed and collected by decanting. The freezing is done to make the humic acid more easily collected (Karpeako and Karaveyev, 1966). The peat is then extracted 5 more times with .05N NaOH, then 3 times with water, and finally air dried and weighed. The weight loss data is in Table 2.

The humic acid samples are then redissolved in .05N NaOH, filtered through a sintered glass funnel and then freeze dried.

Acid Reflux

All the humic acid samples used in the analyses were subjected to hydrolysis in 6N HCl preparatively. This has been found to be beneficial in removing moieties from what can be termed the humic "nucleus" (Riffaldi, 1973, Orlov, 1962). The procedure used was as follows:

Between 3 and 6 grams of dried and finely powdered humic acid is

put into a 125 ml boiling flask. One hundred ml of 6N HCl is added, a condenser inserted on top and the solution is then heated in a mantle. The humic acid/HCl mixture is boiled for approximately 24 hours. It is then collected in a tared sintered glass Buchner funnel. The HCl is saved for absorbance measurement. The residue humic acid is washed with distilled water, dried and reweighed. It is again ground to a fine powder before subsequent chemical analysis.

UV/Visible Spectrophotometry

All UV and visible spectrophotometry done on these samples was done using a Beckman DU monochromator coupled with a Gilford UV-visible source and detector and a Gilford rapid sampler with a one centimeter cell.

The humic acid samples were analyzed by dissolving 10 mg in 50 ml .4N NaOH. The aqueous samples were analyzed by dissolving 10 mg in 50 ml .04N NaOH. The hydrolysates were still in 6N HCl.

The exact wavelengths used were taken from Orlov (1966) and are selected to show the slope of the absorbance curve and not be affected by specific peaks.

Total Acidity

Total acidity of the humic materials is done using the standard method of reaction with $Ba(OH_2$ (Schnitzer 1965). Following is the actual procedure used.

Approximately 100 mg of dried and ground organic matter is put into a 125 ml erlenmeyer flask. To this is added 25 ml of .25N $Ba(OH)_2$ solution. Air is displaced with $N_2$ and the flask is stopped.

It is now stirred for approximately 24 hours at room temperature with a magnetic stirrer. Solution is then poured into a 50 ml syringe and filtered through a .45m millipore filter. Filter cake is washed 3 times with distilled water using a total of 50 ml. Solution is then titrated with .1N HCl using phenolphthalein as endpoint indicator. A blank is determined simultaneously. The acidity is then calculated by:

$$\text{Meq/g Total Acidity} = \frac{(\text{titer of blank} - \text{titer of sample}) \times N \text{ acid} \times 1000}{\text{mg of sample}}$$

The method was checked for accuracy and precision using pure organic acids (salicyclic acid, benzoic acid). The determination was done twice on each sample. The filter cakes are washed and the IR spectra of the reacted materials taken.

Carboxyl Analysis

Carboxyl groups were determined using the method of reaction with $Ca(OAc)_2$ (Schnitzer, 1965). The procedure used was as follows:

To approximately 100 mg of dried and ground organic matter in a 125 ml erlenmeyer flask was added 50 ml of .2N $Ca(OAc)_2$. Air is displaced with $N_2$ and the flask is stoppered. Sample is stirred with a magnetic stirrer at room temperature for 24 hours. Suspension is poured into a syringe and filtered through a .45 mg millipore filter. Flask and filter cake are washed 3 times with distilled $H_2O$ (total 50 ml $H_2O$). The solution is titrated with .1N NaOH to a thymolphthalein endpoint. A blank is determined simultaneously.

Calculations of carboxyl groups is as follows:

meq COOH/g organic matter

$$= \frac{\text{(titer of sample - titer of blank) X N base X 1000}}{\text{mg sample}}$$

Accuracy and precision is again checked using pure organic acids. Filter cake is dried and used for subsequent IR analysis.

Carbonyl Analysis

Carbonyl analysis is done on the samples using a modification of the method outlined by Schnitzer (1972): 100 of finely powdered organic matter is put into a 50 ml glass stoppered reaction vial. Add 8 ml of .25 M dimethylethanol in 2 propanol. Flush with $N_2$. Place in water bath at $70^{o}C$ for 30 minutes with constant magnetic stirring. Cool and titrate potentiometrically with .2N $HClO_4$ in methyl cellosolve. Blank is run without sample. Precision of the method is checked using vanillin and p-hydroxybenzoic acid. Only non-carboxyl carbonyls react. Effect of time of reaction was checked as follows:

| Sample | Time | meq/g c=o | |
|---|---|---|---|
| Mohr Humic | 15 min. | 1.9 | 2.0 |
| | 30 min. | 2.5 | 2.5 |
| | 60 min. | 2.5 | 2.2 |
| 15 Humic | 15 min. | 1.6 | 1.7 |
| | 30 min. | 1.9 | 1.9 |
| | 60 min. | 1.9 | 1.9 |
| 12 Humic | 15 min. | 1.9 | 1.9 |
| | 30 min. | 2.0 | 2.1 |
| | 60 min. | 2.1 | 2.0 |

The potentiometric titrations were done using an Orion Research Model 801 pH meter and Sargent combination pH electrode. It was found

necessary to titrate rapidly to lessen the buffering effect of the organic matter which slowly went into solution during the titration. In practice, $HClO_4$ is added rapidly to a pH of approximately 4.0. It is then added .05 ml per 1/2 min. equilibration time. Endpoint is near pH-2.4.

Infra-red-Analysis

All infra-red spectra were obtained using a Perkin-Elmer model 621 dual-beam spectrometer. Spectra were made at a very slow scan rate (45 min.) with low gain (4.5-5) and attenuation (950-1000). This was done to minimize noise and still detect small peaks or assymmetry in the peaks. It also enables one to look for small shifts in peak location.

Samples were prepared by weighing out air dried material and mixing with 300 mg. of potassium bromide. These two are then intimately ground in a warm mortar. The powder is then dried at least 24 hours over phosphorous pentoxide in a vacuum desicator. Pellets are then pressed in a warm 13 mm. die at 7000 psi for 30 minutes under vacuum. The resulting pellets are translucent brown and show a good dispersal of the organic material. Sample pellets are stored over $P_2O_5$. Except as noted (HCl reflux), 1.5 mg. of sample are used in a pellet.

Considerable attention must be paid to the preparation of the pellets for IR analysis to prevent contamination by moisture (Theng et al., 1966) or alteration of the sample material (Stevenson and Goh, 1974).

Amino Acid Analysis

Total amino acid analysis was done on the aqueous peat extracts using the method described by Stevenson and Cheng (1970). The procedure used was as follows:

Approximately 5 mg of finely powdered humic material is weighed into a 20 ml sample vial and 2 ml of 6N HCl is added. Vial is $N_2$ flushed and sealed by drawing the end in a flame. All sample vials are put into an oven at 110°C. After 24 hours, vials are cooled and opened. The suspension is filtered through Whatman #42 filter paper and the filter cake washed with distilled $H_2O$. filtrate and washings are freeze dried. Filter cake is saved for IR analysis.

After freeze-drying the filtrate is redissolved in $H_2O$ and 6N NaOH is added to pH 11. Tubes with samples are put into a water bath and slowly concentrated with a stream of $N_2$ passing over. After concentration to about 2 ml, the solutions are rediluted to 10 ml with distilled water.

For the analysis, 1/2 ml of the sample solution is added to 1/2 ml of .4 M Na Citrate. This is mixed by swirling, then 2 ml of the mixed colorimetric reagent is added. The tubes are then $N_2$ flushed, capped with aluminum foil and put into a boiling $H_2O$ bath for exactly 30 min. Tubes are removed, cooled with tap water and diluted with 5 ml of 50% EtOH. Optical density is measured with the spectophotometer at 570 nm. Simultaneous with the samples, a series of leucine standards are run to make a calibration curve.

Directions for preparation of the colorimetric reagents are given

by Stenenson and Cheng (1970), with the exception of the Na acetate buffer which is made as follows:

Add 54.4g NaOAc $3H_2o$ to 40 ml distilled water and dissolve in a water bath. Cool. Add 10 ml of glacial acetic acid and make up to 100 ml. Adjust to pH 5.51 with NaOH.

Phenolic OH

The phenolic OH content of the humic molecules is estimated as follows:

Phenolic OH = Total Acidity - Carboxyl

Mixed Solvent Titration

In working with the humic substances it was noticed that $H_2O$/pyridine solution formed a good golvent for the humic material and yielded a titration curve with a clearly defined inflection point. It was thus decided to attempt mixed solvent titrations for all the aqueous samples and compare this data with the acidity data from the acetate method. The titration scheme adopted was as follows:

Approximately 100 mg of dried and powdered organic material is put into a 30 ml glass stoppered reaction vial. Add 5 ml pyridine and 5 ml $H_2O$. Boil for 15 minutes. Cool and titrate. It has been noted (Borggaard, 1974) that organic matter solutions can be titrated fairly accurately if done rapidly. Thus .5N NaOH is added at the rate of 1 drop (.02 ml) per 1/2 minute and the pH is recorded just prior to each addition. Titration was done using a combination pH electrode and Orion pH meter.

The data thus obtained provided smooth curves (Fig. 19-21) with

characteristics of organic acid titrations. The endpoint of the titrations is found by using the same data to plot Δ pH vs. Δ ml. The endpoint is taken as the apex of this curve. There is no indication of more than one endpoint since upon reaction of the humic material with pyridine all carboxyl protons are transferred to pyridine to form pyridinium ions. These are subsequently titrated to give one sharp inflection point.

# CHAPTER III

## RESULTS

In Table 1 are the measured absorbances of the water solutions after several days of extraction time. The absorbance is measured at 425 millimicrons because work here has shown this to be an absorbance maximum for river water organic matter (ROM) solutions. The third column of Table 1 gives the yield of 20 liters of solution after concentrating and freeze-drying of the second extract. The last column is calculated from the preceeding data and gives some idea of how constant $\varepsilon$, the molar extinction coefficient, might be for similar aqueous extracts. Sample #13 was, as indicated, extracted four times. Each time fresh water was added it was allowed to stand, with daily shaking, for two to four weeks to allow as much dissolution as possible. The trend of the data is slowly toward depletion of the water-soluble component as would be expected. It is noted however, that the amount of organic matter which dissolves each time is self-limiting and numerous repetitive extractions would be needed to totally remove this component from the peat.

The amount of humic material that can be extracted from the peat with NaOH solution is considerably greater than the amount that is water extractable. Table 2 gives the weight percent removed by exhaustive extraction. In a study of the composition of various peats from the Okeefenokee (Casagrande 1976) it was found that approximately 40% was extractable from a Minnie's Lake sample with water and NaOH solution.

Table 1

Water Extractable Content

| | A 425 | | | |
|---|---|---|---|---|
| Sample | 1st | 2nd | Mg/l | A/mg |
| 7 cyp | | .150 | 45 | $3.3 \times 10^{-3}$ |
| 8 pra | | .113 | 34 | $3.3 \times 10^{-3}$ |
| 9 pra | | .232 | 93 | 2.5 |
| 11 cyp | | .065 | 19 | 3.4 |
| 12 emerg | .196 | .188 | 70 | 2.8 |
| 13 emerg | .330 | .230(.135,.127) | 70 | 3.4 |
| 14 pra | .112 | .135 | 47 | 2.4 |
| 15 pra | | .370 | 140 | 2.6 |
| C pra | .248 | .365 | 100 | 3.6 |

Table 2

Base Extractable Content

| Sample | % Extracted |
|---|---|
| 7 | 67.2 |
| 8 | 55.7 |
| 9 | 52.4 |
| 11 | 71.4 |
| 12 | 49.9 |
| 13 | 47.3 |
| 14 | 45.5 |
| 15 | 51.7 |
| C | 62.0 |

However, in his analysis of a Chesser Prairie peat, he neglected to analyze a sample from the 50 centimeter depth. The data in Table 2 suggests that it would show near 50% extractable material with these solvents, as samples 14 and 15 are Chesser Prairie samples from .5 meter depth.

Considerable work, both experimental and theoretical, has gone into investigating the application of absorption spectrophotometry in the ultra-violet and visible ranges to the study of humic acids. Orlov (1966) surveys the literature up to that time and concludes that a ratio of absorbances at 474 nanometers and 666 nanometers (E4/E6) is more meaningful than any single select wave length. While he finds this value is directly related to the humic acid content of a solution he cautions that values are not the same for humic acids of different origin. Other workers (Tan and Giddens, 1972) speculate that this value is closely related to the molecular weight or degree of polymerization of the humic molecule. Riffaldi and Schnitzer (1973) consider a decrease in this "color ratio" to indicate an increase in condensation of the humic acid molecule. The physical changes in the fulvic acid molecule that corresponds to the changes in the color ratio have not yet been explained (Schnitzer 1972). This is also certainly true for the aqueous extracts studied here. Tables 3 and 4 contain solution absorbances for the humic substances derived from Okeefenokee peat. The E4/E6 ratios for most of the humic acid solutions fall into the range of brown humic acids which commonly have an E4/E6 ratio of 5 (Orlov 1966).

Extinction coefficients were calculated to be compared with those

Table 3

Solution Absorbance

Humic Acids

| Sample | A425 | A474 | $E_{474}$ .001% 1 cm | A666 | 474/666 |
|---|---|---|---|---|---|
| 7 | 1.32 | .85 | .043 | .14 | 5.67 |
| 8* | 2.39 | 1.49 | .075 | .24 | 6.21 |
| 9 | 2.26 | 1.40 | .070 | .22 | 6.36 |
| 11* | 2.12 | 1.31 | .066 | .18 | 7.28 |
| 12 | 2.20 | 1.37 | .069 | .21 | 6.52 |
| 13 | 1.14 | .75 | .038 | .16 | 4.69 |
| 14* | 2.08 | 1.27 | .064 | .22 | 5.77 |
| 15 | 2.18 | 1.35 | .068 | .22 | 6.14 |
| B1 | 2.46 | 1.79 | .090 | .45 | 4.00 |
| C* | 2.11 | 1.29 | .065 | .20 | 6.45 |
| H* | 1.48 | 1.04 | .052 | .26 | 3.85 |
| Mohr | 1.43 | .91 | .046 | .15 | 6.07 |

*most soluble

10 mg/50 mℓ.4N NaOH

200 ppm

Table 4

Solution Absorbance

Aqueous Extracts

| Sample | Conc. (ppm) | A474 | A666 | 474/666 | A425 (200ppm) |
|---|---|---|---|---|---|
| 7 | 204 | .294 | .032 | 9.2 | .530 |
| 8 | 224 | .366 | .038 | 9.6 | .621 |
| 9 | 250 | .181 | .017 | 10.6 | .289 |
| 11* | 204 | .370 | .039 | 9.5 | .689 |
| 12 | 214 | .628 | .070 | 9.0 | 1.078 |
| 13 | 204 | .636 | .071 | 9.0 | 1.159 |
| 14 | 22 | .038 | .004 | 9.5 | .636 |
| | 202 | .370 | .039 | 9.5 | .708 |
| 15* | 224 | .223 | .032 | 7.0 | .391 |
| B.I. | 102 | .102 | .018 | 5.7 | .349 |
| | 188 | .189 | .036 | 5.5 | .354 |
| C | 206 | .641 | .065 | 9.8 | 1.164 |
| H | 210 | .207 | .023 | 9.0 | .337 |
| M | 208 | .638 | .102 | 6.3 | 1.044 |
| RS36A | 244 | .393 | .032 | 12.3 | .652 |
| RS19 | 198 | .298 | .026 | 11.5 | .573 |

*Did not fully dissolve

of Orlov (1966) for soil humic acids. The values for the peat humic acids seem to fall into two ranges; some are centered around .045 (7, 13, H, M) and the others center around .068. Again the sample from Billy's Island (B.I.) is noticeably different.

The E4/E6 ratios for the aqueous samples are quite high. Schnitzer and Khan (1972) report values of 6.0 to 8.5 for soil fulvic acids. Sample M which was a fulvic acid and sample B.I. which is a soil extract fall into this reported range. The high values for the aqueous peat extracts would suggest that these molecules are less condensed and have a lower molecular weight than humic or fulvic acids.

The absorbance of the peat extracts at 425 nanometers was determined for comparison with similar data on river water organic matter. Reuter and Beck (private communication) have shown a near linear relationship between A425 and concentration of organic material. A linear regression analysis of the data in Table 1 shows an excellent correlation (correlation coefficient of 0.95) between A425 and organic materials in solution.

As stated above, all humic acid samples were hydrolysed for 24 hours in 6 N HCl prior to any further analysis. This procedure removes proteins, peptides, amino acids, sugars, uronic acids, phenols and metals from the humic molecules, (Riffaldi and Schnitzer, 1973). As seen in Table 5 this procedure solubilizes up to 40 per cent of the total weight of the humic acid. Orlov (1962) considers it necessary to remove these moieties from the humic acid in order to better study what he terms the humic nucleus. This, he concludes, is the part of greatest interest in the study of a humic acid.

Table 5

Humic Acid Reflux Data

| Sample | g in | out | % |
|---|---|---|---|
| 7 | 3.50 | 2.48 | 71 |
| 8 | 3.84 | 2.36 | 61 |
| 9 | 3.00 | 2.03 | 68 |
| 11 | 4.87 | 3.17 | 65 |
| 12 | 5.96 | 3.52 | 59 |
| 13 | 4.97 | 3.67 | 74 |
| 14 | 6.85 | 3.73 | 63 |
| B.I. | 4.89 | 3.08 | 63 |
| C | 5.93 | 3.73 | 63 |
| H | N.A. | | |
| 15 | 4.97 | 3.09 | 62 |
| M | 5.10 (6.35) | 3.85 (4.59) | 75 (72) |

Table 6 shows the elemental analysis of one of the humic acid samples (M) before and after the HCl reflux. The extreme change in the C/N ratio is due to removal of protein fragments by hydrolysis of peptide bonds. The high C/H ratio of the humic acid after reflux is an indication that what is left is a more aromatic residue.

Table 7 shows the E4/E6 ratios for the material that is mobilized by the hydrolysis of the humic acid. While the interpretation of ratios of this magnitude is not possible it is reasonable to assume that simple removal of this fraction would lower the E4/E6 ratios of the residual humic acid. Therefore decrease in this ratio observed by Riffaldi and Schnitzer (1973) does not necessarily imply condensation reactions affecting the humic nucleus.

Tables 8 and 9 contain the results of the chemical analyses of the peat humic fractions studied. The Okefenokee peat humic acid falls into the ranges given by Schnitzer and Kahn (1972) for soil humic acids. Those ranges are: total acidity 5.8 - 10.2 meq/g carboxyl 1.5 - 4.7 meq/g, phenolic OH 2.1 - 5.7 meq/g and carbonyl 0.9 - 5.2 meq/g.

The aqueous extracts of the peats were expected to more closely resemble fulvic acids. The ranges of functional group content given by Schnitzer and Kahn (1972) for soil fulvic acids are: total acidity 11.8 - 14.2 meq/g, carboxyl 8.5 -9.1 meq/g, phenolic OH 1.7-5.7 meq/g and carbonyl 1.1 - 3.1 meq/g. In comparing these values to those in Table 9 one finds that the Okefenokee samples are below the normal in carboxyl content and in total acidity. This is also true of sample M which is a peat fulvic acid.

Table 6

Elemental Analysis

M Humic Acid

| | Original | After HCL Reflux | |
|---|---|---|---|
| Element | %(wt.)found | Ash-free | %(wt.) found |
| C | 54.50 | 55.23 | 60.52 |
| H | 5.46 | 5.53 | 4.93 |
| N | 2.57 | 2.71 | 1.16 |
| O* | 36.05 | 36.53 | 33.39 |
| Ash | 1.32 | | 0.0 |
| C/H | | .84 | 1.03 |
| C/O | | 2.01 | 2.41 |
| C/N | | 24.2 | 63.0 |

*by difference

Analysis by Atlantic Microlabs.

Table 7

Solution Absorbance

Humic Hydrolysates in 6N H-Cl

| Sample | A425 | A474 | A666 | 474/666 |
|---|---|---|---|---|
| 7 | .755 | .276 | .010 | 28 |
| 8 | 1.358 | .454 | .016 | 28 |
| 9 | 1.617 | .639 | .105 | 6 |
| 11 | 1.328 | .563 | .045 | 12 |
| 12 | 1.461 | .545 | .035 | 16 |
| 13 | .670 | .280 | .012 | 22 |
| 14 | 1.850 | .660 | .033 | 20 |
| H | 3.620 | 2.105 | .140 | 15 |

Table 8

Functional Group Analyses

Humic Acids

| Sample | Total Acidity | Carboxyl | Phenyl-OH | Carbonyl |
|---|---|---|---|---|
| 7 | 6.4 | 2.7 | 3.7 | 2.0 |
| 8 | 7.3 | 3.3 | 4.0 | 2.5 |
| 9 | 7.1 | 3.3 | 3.9 | 2.5 |
| 11 | 7.2 | 3.3 | 4.0 | 2.3 |
| 12 | 6.9 | 3.2 | 3.7 | 2.1 |
| 13 | 6.7 | 3.0 | 3.7 | 2.8 |
| 14 | 7.8 | 2.9 | 5.0 | 1.8 |
| 15 | 7.3 | 2.8 | 4.5 | 1.9 |
| B.I. | 7.3 | 3.9 | 3.4 | 3.1 |
| C | 6.6 | 3.1 | 3.5 | 2.4 |
| H | 4.1 | 2.8 | 1.3 | 1.8 |

The Δ column in Table 9 reports the difference in total acidity before and after the desalting (see procedures). This gives some measure of the number of exchange sites still available if the molecule were to enter the river system. It is found that as much as 50% of the available sites were already complexed at the time of extraction. Working with samples from the Okefenokee, Casagrande (1976) found the major cations in the peat to be Fe, Ca, Mg, Na and K.

The carboxyl content of the aqueous samples was determined by two methods; reaction with calcium acetate followed by titration of the acetic acid formed and by titration with NaOH after dissolution in pyridine-water. Results from both methods are tabulated in Table 9. The calcium acetate method is found to yield consistently higher results, but agreement between the two methods is still quite good. It is suggested that this difference is due to a change in the pKa of ortho phenolic protons upon formation of calcium-carboxylate bonds.

The results of the carbonyl analysis, also in Table 9, show very little difference, particularly among the aqueous samples. The samples from totally different sources (H, B.I. and RS 36A) are the only ones showing much deviation from an average of 2.0 meq/g for the humic acids and 2.5 meq/g for the aqueous extracts.

The total amino acid content of the aqueous extracts are also found to provide little basis for differentiating between samples. Beck et al. (1974) reported values of 3.5 - 5.5% for river water organic matter. Casagrande and Given (1974) found 3 to 8% amino acids in Everglades peats. The values herein for Okefenokee peats are consistent with these results.

Table 9

Functional Group Analyses

Aqueous Extracts

| Sample | Total Acidity | | | Carboxyl | | Phenolic OH | |
|---|---|---|---|---|---|---|---|
| | Initial | Desalted | Δ | Acetate Method | Pyrid/$H_2O$ | 1* | 2* |
| 7 | 3.9 | 8.1 | 4.2 | 5.7 | 4.5 | 2.4 | 3.6 |
| 8 | | 7.3 | | 5.0 | 4.6 | 2.3 | 2.7 |
| 9 | 4.7 | 7.0 | 2.3 | 5.4 | 4.5 | 1.6 | 2.5 |
| 11 | | 7.9 | | 5.9 | 5.0 | 2.0 | 2.9 |
| 12 | 5.1 | 7.2 | 2.1 | 4.3 | 3.7 | 2.9 | 3.5 |
| 13 | 6.0 | 8.6 | 2.6 | 5.1 | 4.9 | 3.5 | 3.7 |
| 14 | 3.8 | 7.0 | 3.2 | 5.2 | 4.2 | 1.8 | 2.8 |
| 15 | 4.3 | 7.1 | 2.8 | .6 | | 6.5 | |
| B.I. | 5.9 | 8.2 | 2.3 | 5.6 | 4.7 | 2.6 | 3.5 |
| C | 6.2 | 7.5 | 1.3 | 4.6 | 4.3 | 2.9 | 3.2 |
| M | 7.0 | 7.1 | .2 | 4.1 | 4.0 | 3.0 | 3.1 |
| H | 1.5 | 4.2 | 2.7 | 2.9 | 2.9 | 1.2 | 1.2 |
| RS36A | | 10.5 | | 6.0 | 5.2 | 4.5 | 5.3 |

* Column 1 calculated using acetate method values, column 2 calculated using pyrid/$H_2O$ titration values.

Table 10

Functional Group Analyses

Aqueous Extracts

| Carbonyl | Total Amino Acid |
|---|---|
| 2.8 | 5.7 |
| 2.3 | 6.5 |
| 2.4 | 6.3 |
| | 5.5 |
| 2.8 | 6.7 |
| 2.6 | 5.9 |
| 2.3 | 5.4 |
| -1.0 | 6.3 |
| 2.8 | 6.0 |
| 2.4 | 5.7 |
| 1.5 | 0.2 |
| 3.6 | 4.8 |

Infra-red Spectra

In the past 20 years, scanning infra-red spectrophotometry has become one of the most valuable tools of analytical chemistry. With it, it is possible to discern not only the types of bonds within a molecule but, frequently, to make inferences as to the environment of the absorbing moiety. For example, Schnitzer (1975) has used the absorption region of OH in hydrogen-bonded carboxyl groups (2700-2400 $cm^{-1}$) for a quantitative estimation of this functional group. Yekaterinina (1969) has shown that it is possible to discern between conjugated and non-conjugated carbonyl groups by taking advantage of the fact that the carbonyl absorption shifts from 1700-1715 $cm^{-1}$ to 1640-1680 $cm^{-1}$ upon conjugation.

The major problem in the use of infra-red spectrometry in the study of humic substances is the wide diversity of environment possible for each specific moiety. This leads to a considerable increase in the band widths and consequent overlap of absorption. Consequently classification of humic spectra is based on general types of patterns (Stevenson and Goh, 1971) as opposed to the highly specific pattern of a pure low molecular weight compound.

The infra-red spectra of a humic acid before and after hydrolysis and of the HCl solute are shown in Figure 2. Absorption in the 3400 $cm^{-1}$ region is due to O-H and N-H stretch. The acid hydrolysis removes protein residues as evidenced by the decrease in absorption at 1540 $cm^{-1}$ and also polysaccharide components as evidenced by reduced absorption at 1050 $cm^{-1}$. The removal of these components probably accounts for the reduction in absorption in the 3400 $cm^{-1}$ region.

The other prominent change is a decrease in absorption at 1625 $cm^{-1}$

(aromatic C=C, C=C conjugated with C=O). It is possible that the absorption at 1625 $cm^{-1}$ is due to the hydrogen bonded enol form of aliphatic components of the humic molecule. Acid hydrolysis of these ketones would cleave off carboxylic acid molecules. The absorption at 1725 $cm^{-1}$ shows a small increase in agreement with this interpretation.

The IR spectrum of the material left in the HCl solution after the reflux of the humic acid is also shown. In the region above 1400 $cm^{-1}$ the spectrum is very similar to that of the humic acid. The broad absorption centered around 1100 $cm^{-1}$ is probably due to the C-O stretch of sugars removed from the humic acid (Riffaldi and Schnitzer 1973, Rogers 1965). The two peaks in the 800-900 $cm^{-1}$ region are possibly due to phenolic hydrogen bending. Hydrolytic removal of phenolic acids from humic acids has been reported by Riffaldi and Schnitzer (1973) and Anderson et al., (1978). The peaks may also be due to pyranose and furanose rings. This possibility is supported by the large polysaccharide absorbances at 1100 $cm^{-1}$ and, as purposed, at 3400 $cm^{-1}$. The small peak evident at 1540 $cm^{-1}$ in Figure 2 is probably due to N-H of peptide bonds.

In looking at the spectra of the humic acids (Figs. 3-5) derived from Okefenokee peats, one sees that they most closely resemble those classed as Type I by Stevenson. The most notable deviation from the Type I is considerably more C-H absorption at 2920 $cm^{-1}$ and 2840 $cm^{-1}$. These absorptions are due to aliphatic chains in the peat humic acids. This observation suggests that these molecules are less aromatic than is typical of soil humic acids. One's attention is also drawn to the peaks

at 700 $cm^{-1}$ and 790 $cm^{-1}$ in sample H (Fig. 5). These absorption bands are caused by C-H bending (out-of-plane) and could be indicative of aromatic protons on substituted benzene rings. This could be due to a lower degree of saturation in the humic molecule or, as this is a precipitated estuary sample, these could be absorptions of an inorganic component.

The IR spectra of the purified (desalted) aqueous extracts fall into both Type I (as did the humics) and Type II (#8, 9, 11, 14, 36A, H, B.I.). The Type II samples show considerably more carbonyl absorption at 1720 $cm^{-1}$.

The absorption centered at 1725 $cm^{-1}$ is generally assigned to C=O stretch of carboxylic acids (Table II). The spectra of the calcium and barium salts of Type II samples generally show shoulders in this region centered about 1710 $cm^{-1}$. This is considered to be indicative of non-conjugated (ketonic and aldehydic) carbonyls (Yekaterinina 1969, Stevenson, 1971). However, as there is no correlation between the intensity of this absorbance and the results of the carbonyl analysis (Table 10), contributions by other absorbing moieties (e.g. ester) should be considered.

Upon comparing the spectral patterns before and after desalting only one major and consistent trend is observed. This is the decrease of absorption at 1400 $cm^{-1}$ and $\sim$ 1600 $cm^{-1}$ with a concomitant increase of absorption at 1720 $cm^{-1}$, indicating the conversion of carboxylate anion of carboxylic acids (Stevenson 1971). The extent of this change is estimated analytically in Table 9 where it is found that 25-40% of the carboxyl groups are in salt from when first extracted from

the peat. These concomitant spectral changes can again be noted in the spectra of the calcium and barium salts. After salt formation the 1725 $cm^{-1}$ peak very nearly disappears while the peaks at 1600 $cm^{-1}$ (asymmetric stretch) and 1400 $cm^{-1}$ (symmetric stretch) are seen to grow. This indicates a quantitative reaction with the reagents.

After desalting one observes a slight increase in absorption in the 1200 $cm^{-1}$ region. This observation is consistent with Stevenson's assignment of C-O stretch (1200 $cm^{-1}$) and O-H deformation (1230 $cm^{-1}$) of COOH groups. As pointed out by Flaig (1975) the C-O stretch mode of esters, ethers and phenols also have characteristic absorbances in this region. A decrease in absorption in this region is noted for both barium and calcium salts.

RS36 (Fig. 18)

This sample, a river organic matter sample, has been thoroughly investigated by Martin (1974) and was included herein for comparative purposes. Its spectral pattern is, as concluded by Martin, of Type II although the peak at 1725 $cm^{-1}$ is not nearly as pronounced as it is in some of Stevenson's samples (1971). As implied by the substantial absorption in this region, the carboxyl content of this sample is high (Table 9). Upon desalting the peak at 1725 $cm^{-1}$ grows considerably, indicating that in the river some acid sites are complexed. Upon salt formation a shoulder is left circa 1700 $cm^{-1}$ indicating a high carbonyl content. This is confirmed by chemical analysis (Table 10). A second point of interest, also to be noted in other spectra (Fig. 8) is the loss of a broad shoulder at 2700-2400 $cm^{-1}$ upon salt formation. This shoulder has been shown by Schnitzer and Griffith (1975) to be

absorption by hydrogen bonded carboxyl groups. Upon acid reflux of this and other samples, a decrease is noted in the absorption at 1725 $cm^{-1}$. This has been explained as acid-induced decarboxylation in humic acids by Riffaldi and Schnitzer (1973) and such an effect could be advanced to explain the loss here. The peak seen at circa 800 $cm^{-1}$ has been assigned (Beck et al., 1974) to the C-H deformation mode of aromatic components.

M Fulvic (Fig. 15)

This sample is the only fulvic acid sensu stricto i.e., the material remaining in solution after acidification of a basic soil organic matter extract. While ROM is often classified as a fulvic acid (Packham 1964, Christman and Ghassemi, 1966) it is found to be significantly different. The peat from which this sample was extracted was from an island fringe and hence the parent biota are classed as emergent aquatic (Cohen 1974) and should be compared directly to the emergent aquatic aqueous extracts (Figs. 10 and 11). A most striking different is the considerable absorption in the 2900 $cm^{-1}$ region. These bands are not well developed in the aqueous extracts but are more pronounced in the humic acids of the peats. The aqueous extracts are clearly not the same as the fulvic acid sample. One would suspect that the fulvic acid sample contains a considerable amount of material that would not initially be water soluble but is perhaps kept in solution as colloids when the basic extract is acidified. The E4/E6 ratio of this sample is low (Table 4) suggesting a higher degree of condensation than either the aqueous extracts or the river organic matter sample. Again, in the functional group analyses (Table 9) this sample is found to

contain lesser amounts of functional groups than the other samples (except H). A second major difference is the occurance of a band at around 1540 $cm^{-1}$ (N-H bending) which disappears after hydrolysis with HCl. This peak is not observed in any other spectra herein, however it is common in fulvic acid spectra and is used as an identifier for Type III IR spectral patterns (Stevenson 1971).

H Aq. (Fig. 17)

This sample was extracted from a mass of sand and organic matter lying on the bank of the Crooked River Estuary. In it's original extracted form, it has only minimal residual absorption in the 1700 $cm^{-1}$ region. This is indicative of a low carbonyl content, which is confirmed in Table 10. It also implies that the carboxyl moieties, which appear at 1720 $cm^{-1}$ upon desalting, are nearly all converted to carboxylate ions. This is confirmed by the fact that sample H, while having the lowest total acidity (Table 9), has the highest percent increase ( $\Delta$ column) upon desalting. At least two peaks are found in this sample that are not so clearly resolved in any other spectra. The absorption in the 1150 $cm^{-1}$ region falls into the C-H "fingerprint" region. Exact assignment of the absorbing moiety is not possible. The existence of these two well defined bands points to a high concentration of rather similar structural units. After desalting, the sample looks a bit more like the others, but these two absorption bands are observed in all reaction products. The existence of a non-humic component in this extract can not be ruled out.

Samples 9 and 15

In these two samples a group of peaks is found at 1430 $cm^{-1}$,

875 $cm^{-1}$ and 710 $cm^{-1}$. These three bands are assigned by Vinkler et al. (1976) to an inorganic carbonate co-extract. In these two samples the C-O stretch band of carboxyl moieties at 1725 $cm^{-1}$ has almost vanished, indicating that a high percentage of this moiety occurs as carboxylate ion.

In sample 15 one finds, after desalting, that a small peak is still visible at 875 $cm^{-1}$ and also the peak at 1725 $cm^{-1}$ has not appeared. This is probably due to the whole desalting capacity of the column being used to remove most of the carbonate and consequently the carboxylate moieties were not protonated. The wet chemistry analytical date demonstrate that sample 15 is reacting differently than the other humic substances. It is suspected that the incomplete desalting is the cause. Sample 15 also shows considerable absorption in the 2900 $cm^{-1}$ region. In this respect it resembles the fulvic acid sample (M). This absorption is indicative of a high aliphatic character to this sample.

ROM Salts

In comparing the I.R. spectra of the calcium and barium salts of the aqueous extracts two subtle but significant differences can be noted (Figs. 10, 11 and 17). The calcium salt spectra show a shoulder near 1730 $cm^{-1}$. This is assigned to the C=O stretch of ketones, quinones and possibly lactones. The barium salts are noticeably lacking in this absorption which should not disappear after simple salt formation. It is further noted that $V_{as}COO^-$ is at 1585 $cm^{-1}$ for calcium salts and at 1560 $cm^{-1}$ in the spectra of the barium salts. Vinkler et al. (1976) in a study of carefully prepared humic acid salts found values of 1580 $cm^{-1}$ and 1595 $cm^{-1}$ for the calcium and barium salts respectively.

Thus in both instances there is an indication that the $Ba(OH)_2$ is doing more than simply reacting to form carboxylic acid salts. It is suspected, on the basis of this evidence, that the strong base (barium hydroxide) is inducing Claisen condensation reactions within the humic molecule.

Table 11

| $Cm.^{-1}$ | Assignment | Source |
|---|---|---|
| 3450-3300 | Hydrogen-bonded OH groups, OH intermolecular-bonded OH | Flaig (75) |
| 3077-3030 | Aromatic C-H stretching | Flaig (75) |
| 2950-2850 | Aliphatic C-H, C-H, C-H, stretching | Flaig (75) |
| 2850-2500 | Carboxylate ion | Flaig (75) |
| 2700-2400 | H-bonded COOH | Schnitzer & Griffith (75) |
| 1725-1640 | C=O stretching of carboxylic acids, cyclic & acyclic aldehydes and ketones | Flaig (75) |
| 1640-1680 | Quinones | Yekaterinina (69)<br>Stevenson (72) |
| 1600 | C=O chelated | Stevenson (72) |
| 1650 & 1540 | N-H of peptides, amides | Otsuki, Stevenson (71) |
| 1640-1585 | C=C stretching vibration of double bonds in cyclic & acyclic compounds, benzene rings, substitution | Flaig (75) |
| 1575 | Carboxyl ion (metallic derivatives of chelated carbonyl groups) | Flaig (75) |
| 1540 | $NO_2$ vibrations of nitro groups (mainly in humic acids prepared by nitric acid oxidation from coals) | Flaig (75) |
| 1515 | C=C Stretching vibration of benzene, pyridines, etc., benzene ring substitution secondary amines | Flaig (75) |
| 1470-1420 | Aliphatic C-H deformation | Flaig (75) |
| 1430,873,712 | Carbonate (inorganic) | Vinkler (76) |
| 1400 | OH deformation, C-O stretch of phenol hydroxyls | Stevenson (71) |
| 1390-1332 | Salts of carboxylic acids | Flaig (75) |
| 1280-1137 | C-O stretching of esters, ethers, and phenols | Flaig (75) |
| 1230 | OH deformation | Tan & Giddens (72) |
| 1200 | C-O stretch of COOH | Stevenson & Goh (72) |
| 1052 | Carbohydrate | Flaig (75) |
| 1040 | Ethers, alcohols | Maksimova (73) |
| 1025 | Si-O-Si vibration of silicates | Flaig (75) |
| | C-H bending of aromatics | Beck (74) |

# CHAPTER IV

## CONCLUSIONS

It was originally hoped that it would be possible to identify the precursor biota by some characteristic of the humic substance; this was not found possible. Indeed, the homogeneity throughout the swamp is striking.

The obvious differences between the aqueous peat extracts and the river organic matter samples indicates some sort of fractionation takes place during the extraction and subsequent flushing from the swamp proper.

While the aqueous molecules do have a considerable cation content, the potential extent of complexing/chelating with cations while in the river is considerable.

The organic molecules are mobilized from the peat by incoming ground waters. The moieties dissolved are partially in salt form. This probably increased their solubility.

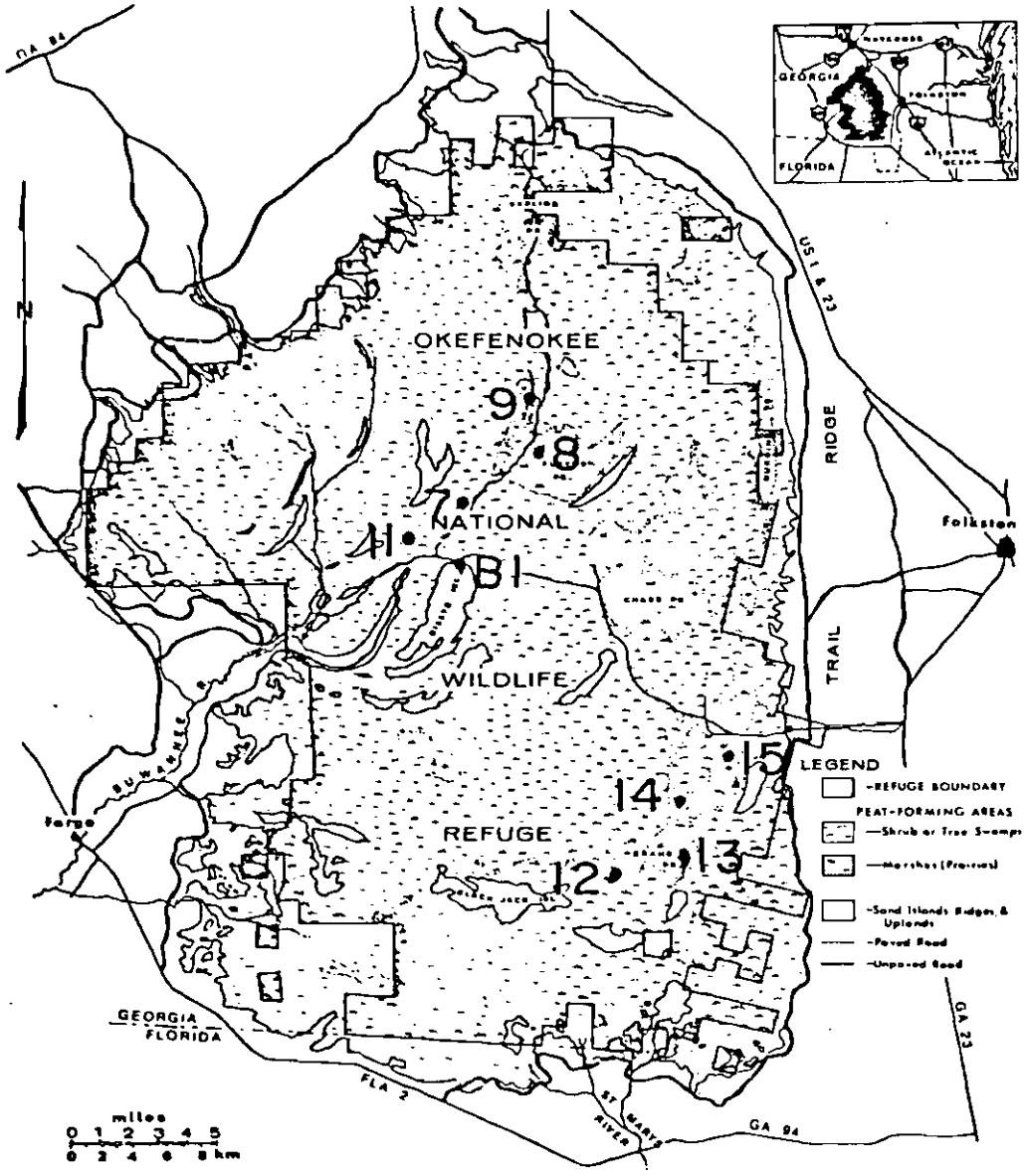


Figure 1. Location and physiographic features of the Okefenokee Swamp.

Table 12

Abbreviations on IR Spectra

| | |
|---|---|
| Aq | material readily soluble in distilled water. |
| Ba | barium salt of organic material; obtained by reaction with $Ba(OH)_2$ (total acidity determination). |
| Ca | calcium salt; obtained by reaction with calcium acetate (carboxyl analysis). |
| CO | oxime derivative of organic material; obtained by reaction with hydroxylamine (carbonyl analysis). |
| D | desalted. |
| F | fulvic acid. |
| H | humic acid. |
| HCl | material remaining as solid after reflux in 6 N HCl. |
| R | refluxed sample (6 N HCl). |
| S | material remaining in solution in 6 N HCl after reflux. |

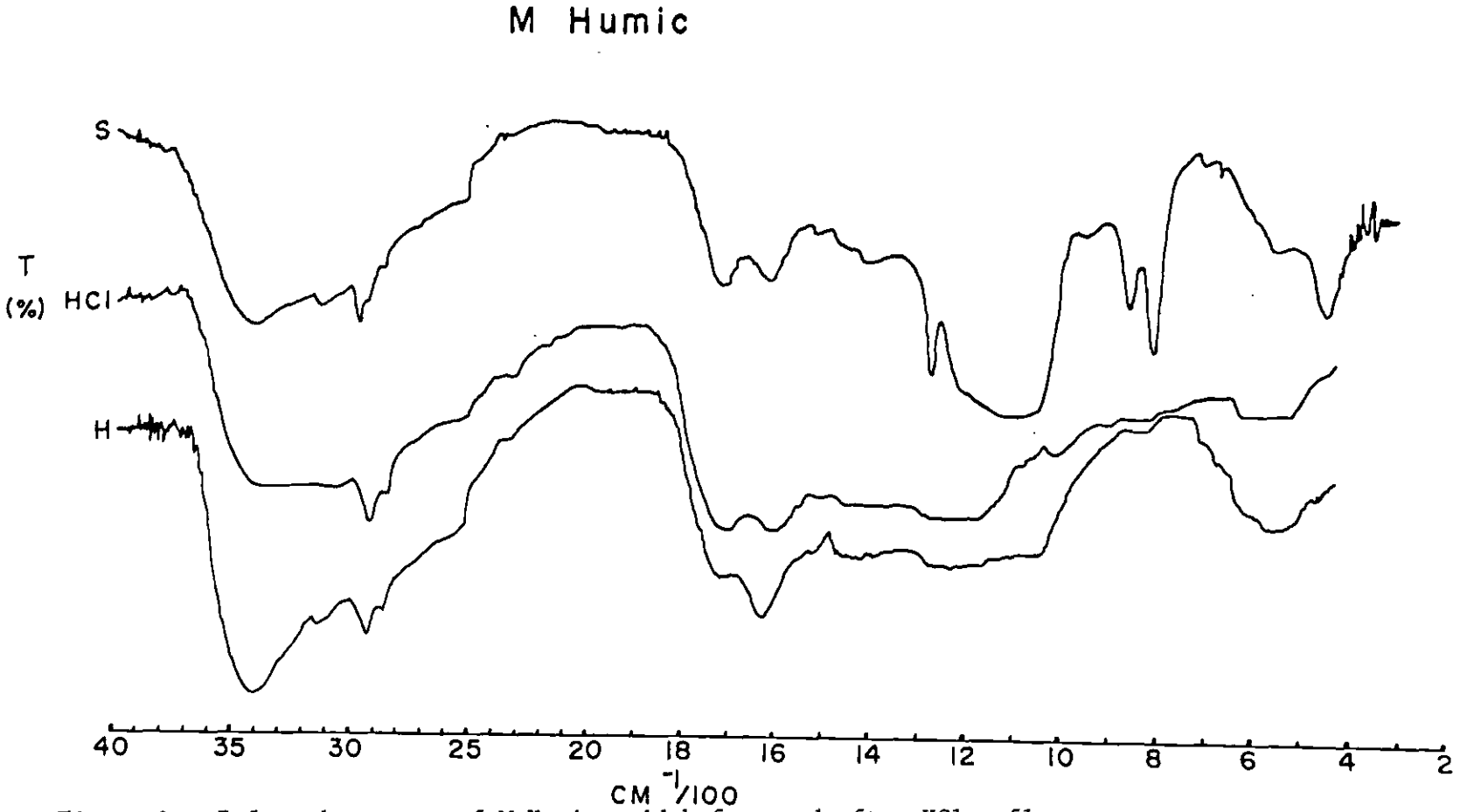


Figure 2. Infrared spectra of M Humic acid before and after HCl reflux.

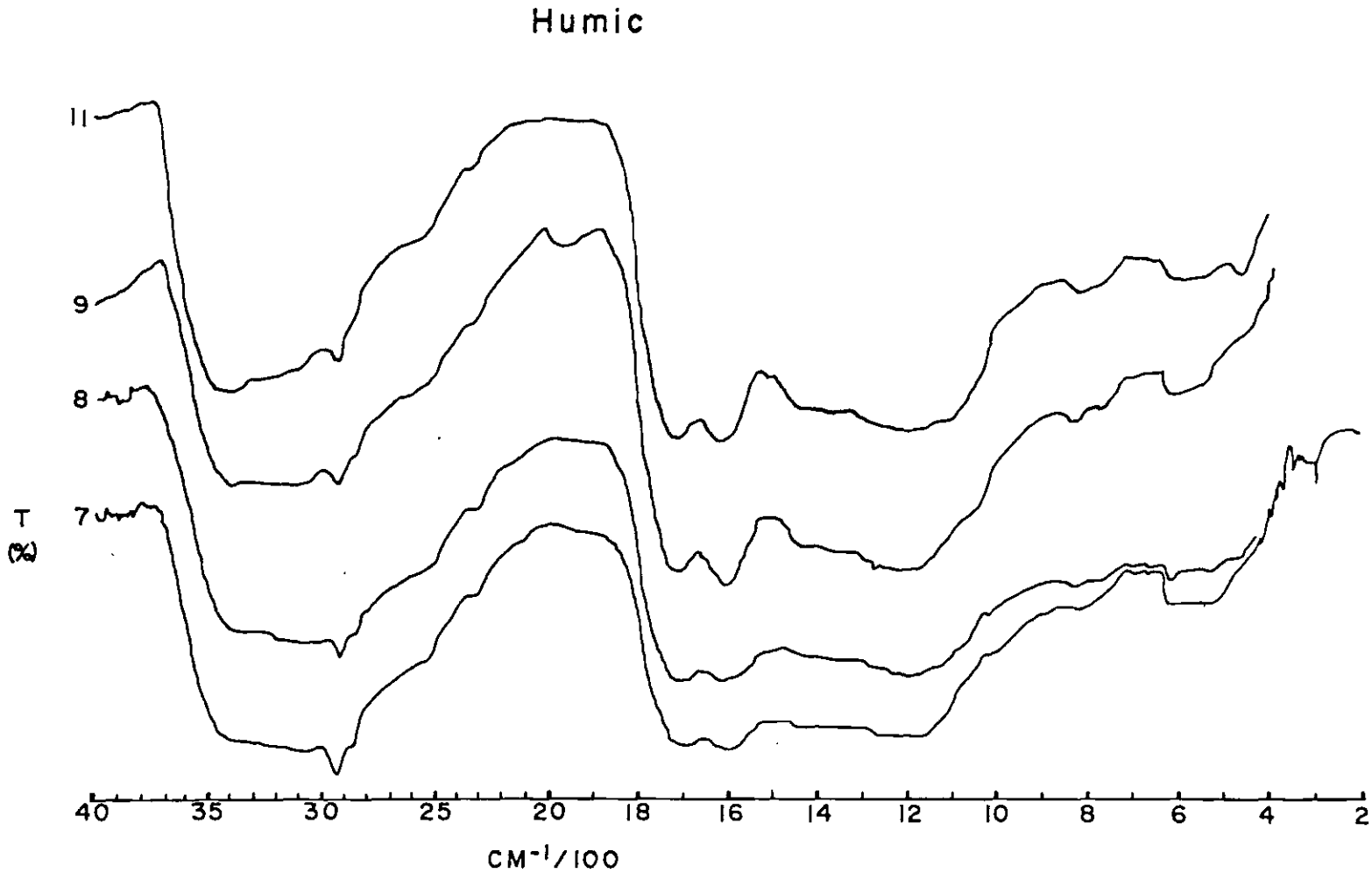


Figure 3. Infrared spectra of Okefenokee humic acids.

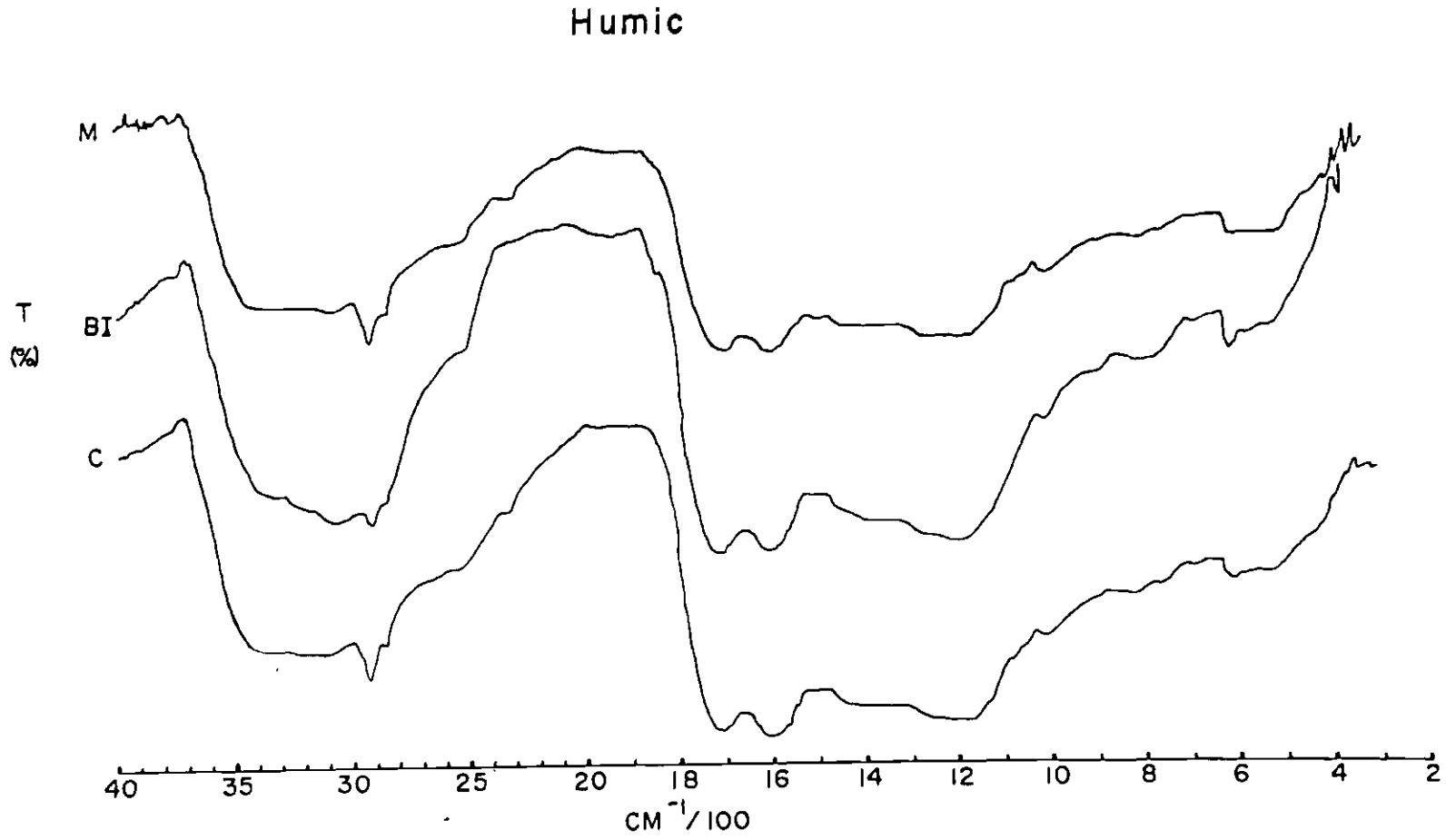


Figure 4. Infrared spectra of Okefenokee humic acids. (Continued)

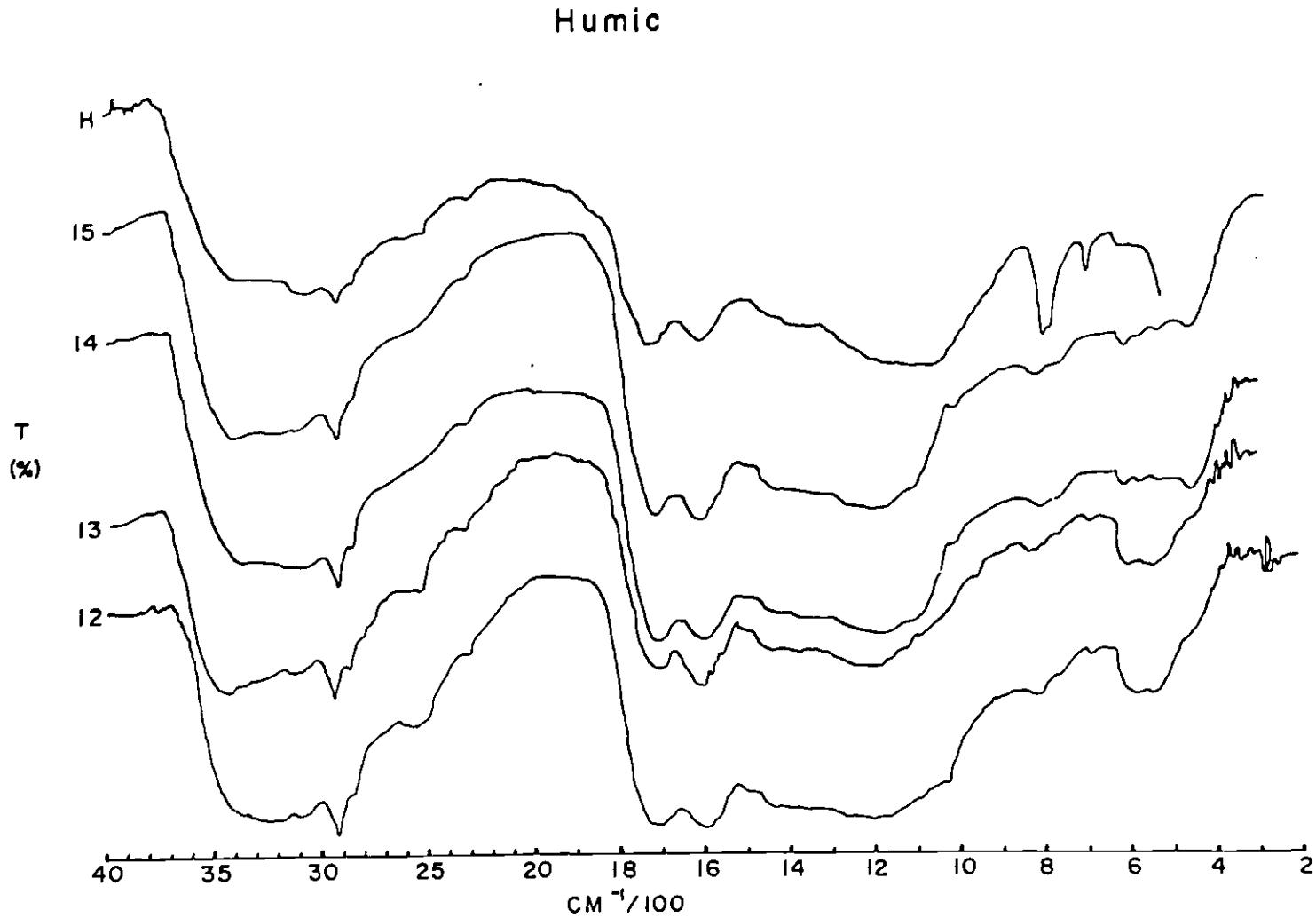


Figure 5. Infrared spectra of Okefenokee humic acids. (Continued)

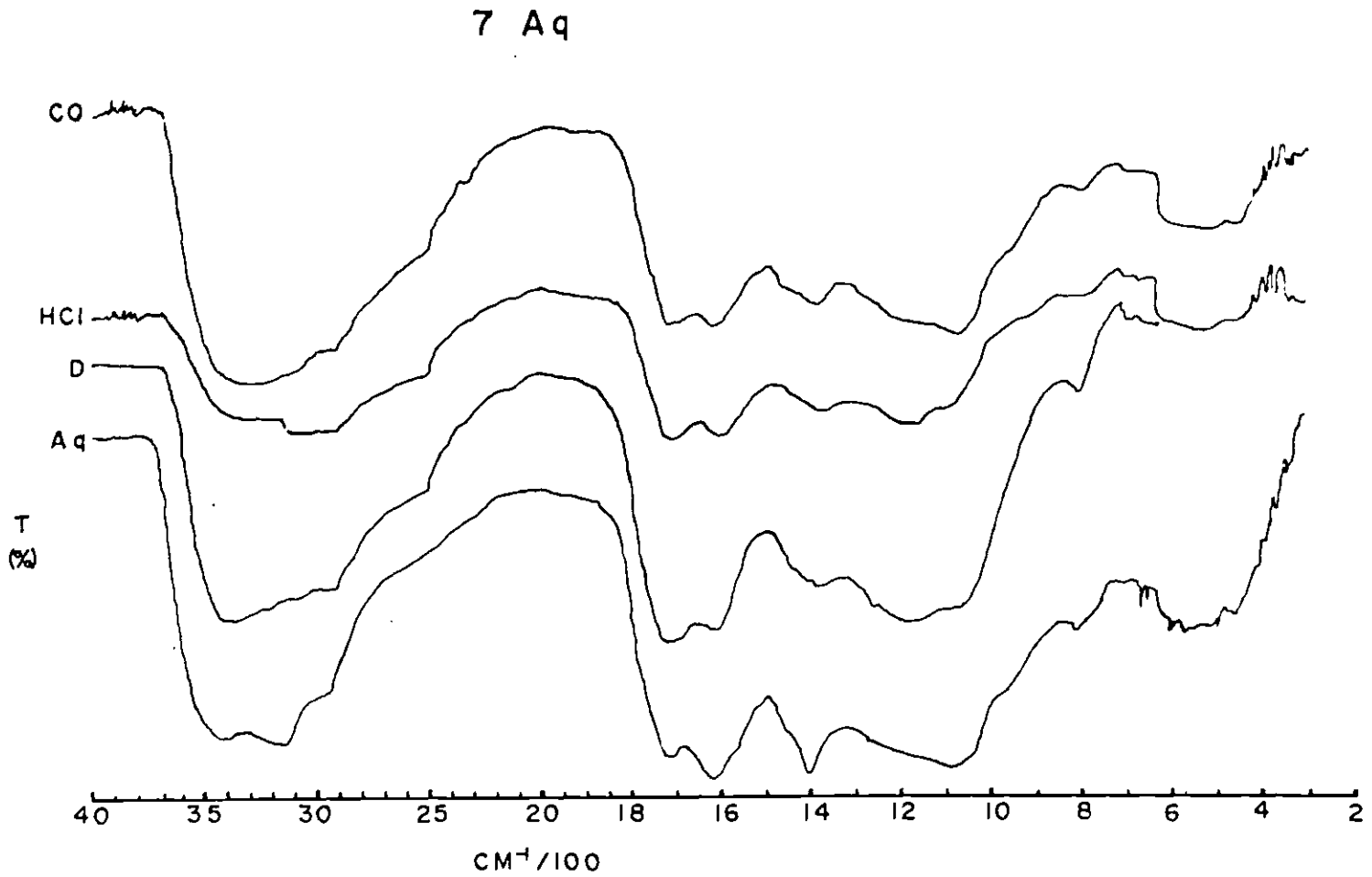


Figure 6. Infrared spectra of aqueous extract of Sample #7 and derivatives.

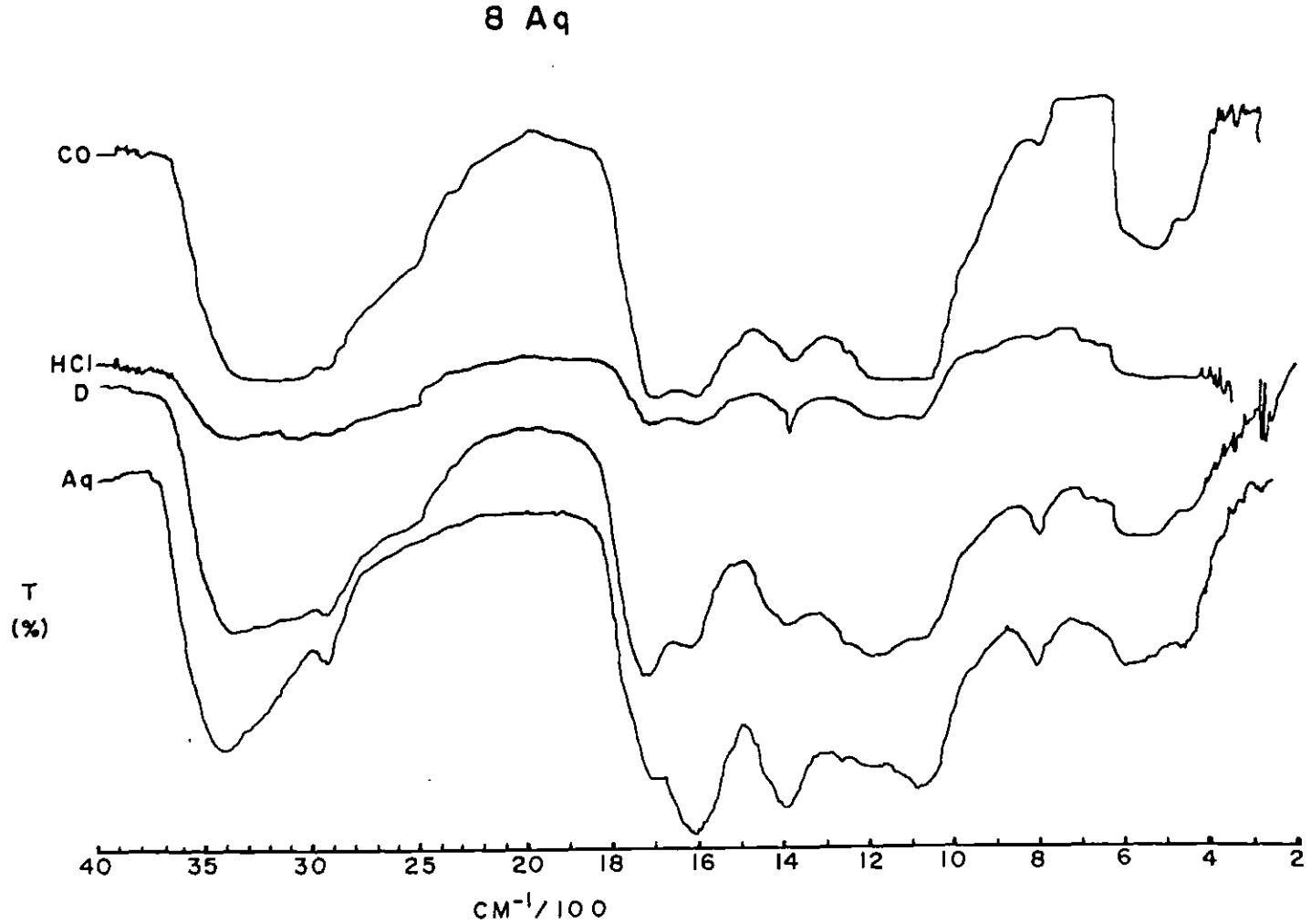


Figure 7. Infrared spectra of aqueous extract of Sample #8 and derivatives.

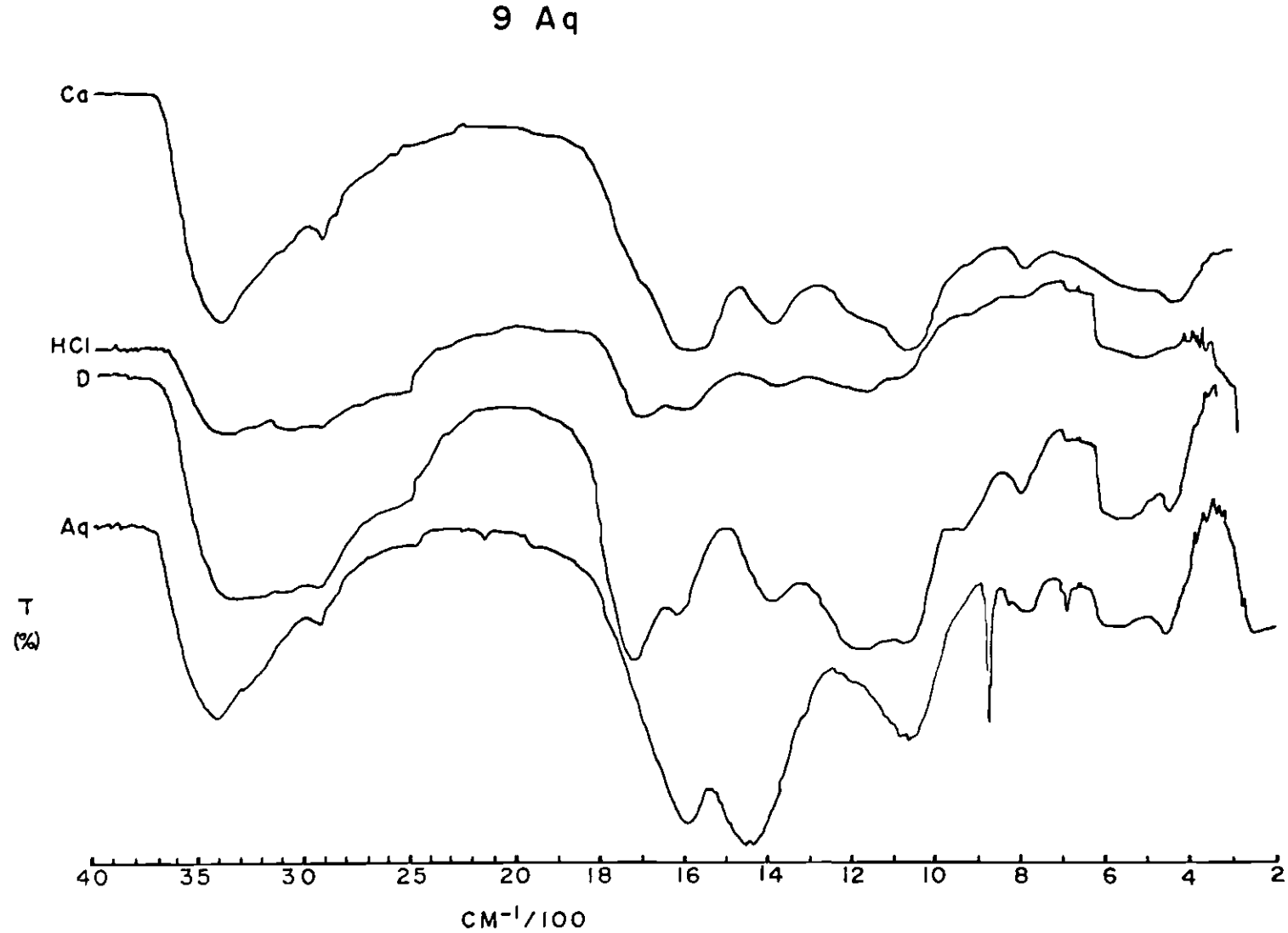


Figure 8. Infrared spectra of aqueous extract of Sample #9 and derivatives.

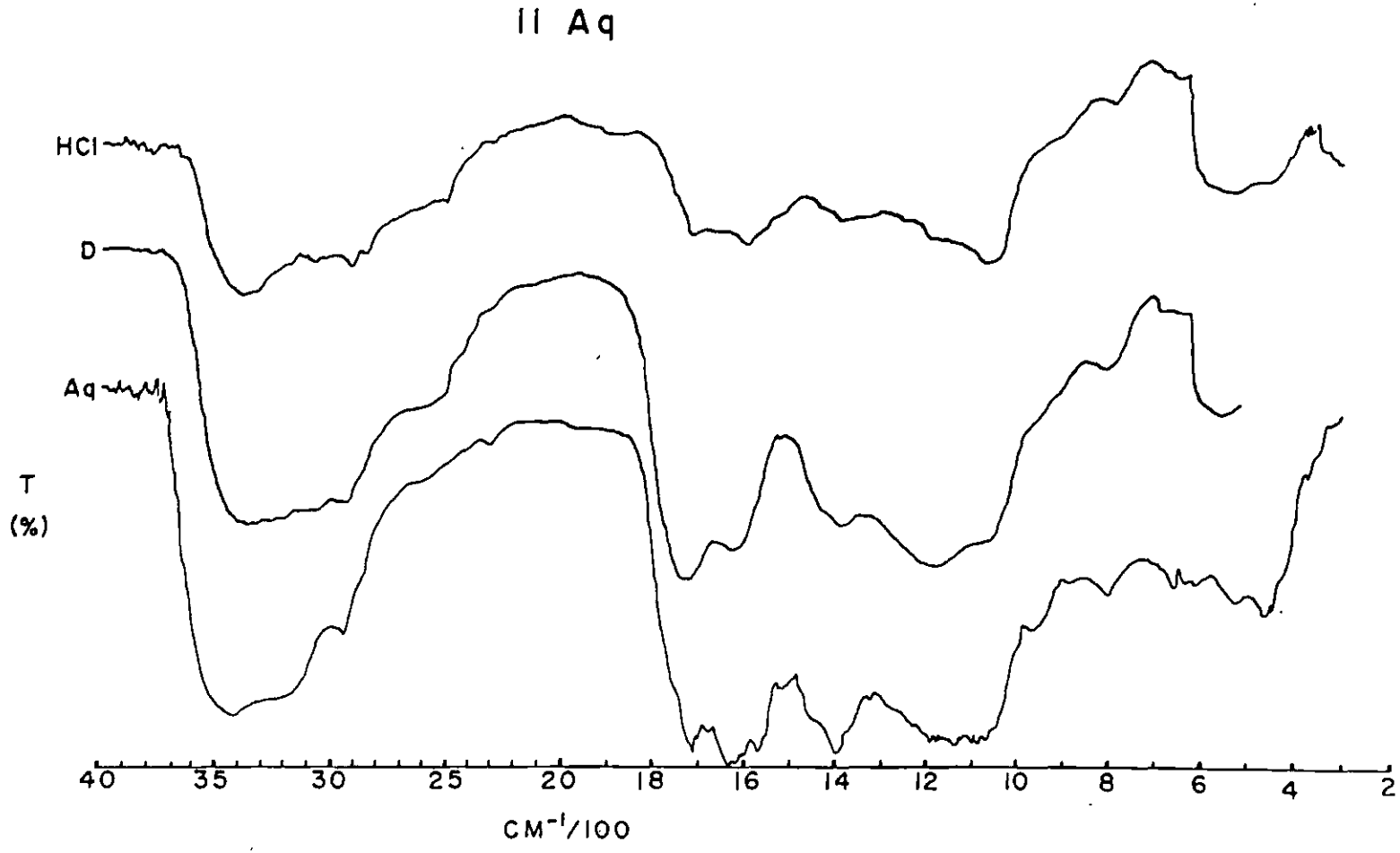


Figure 9. Infrared spectra of aqueous extract of Sample #11 and derivatives.

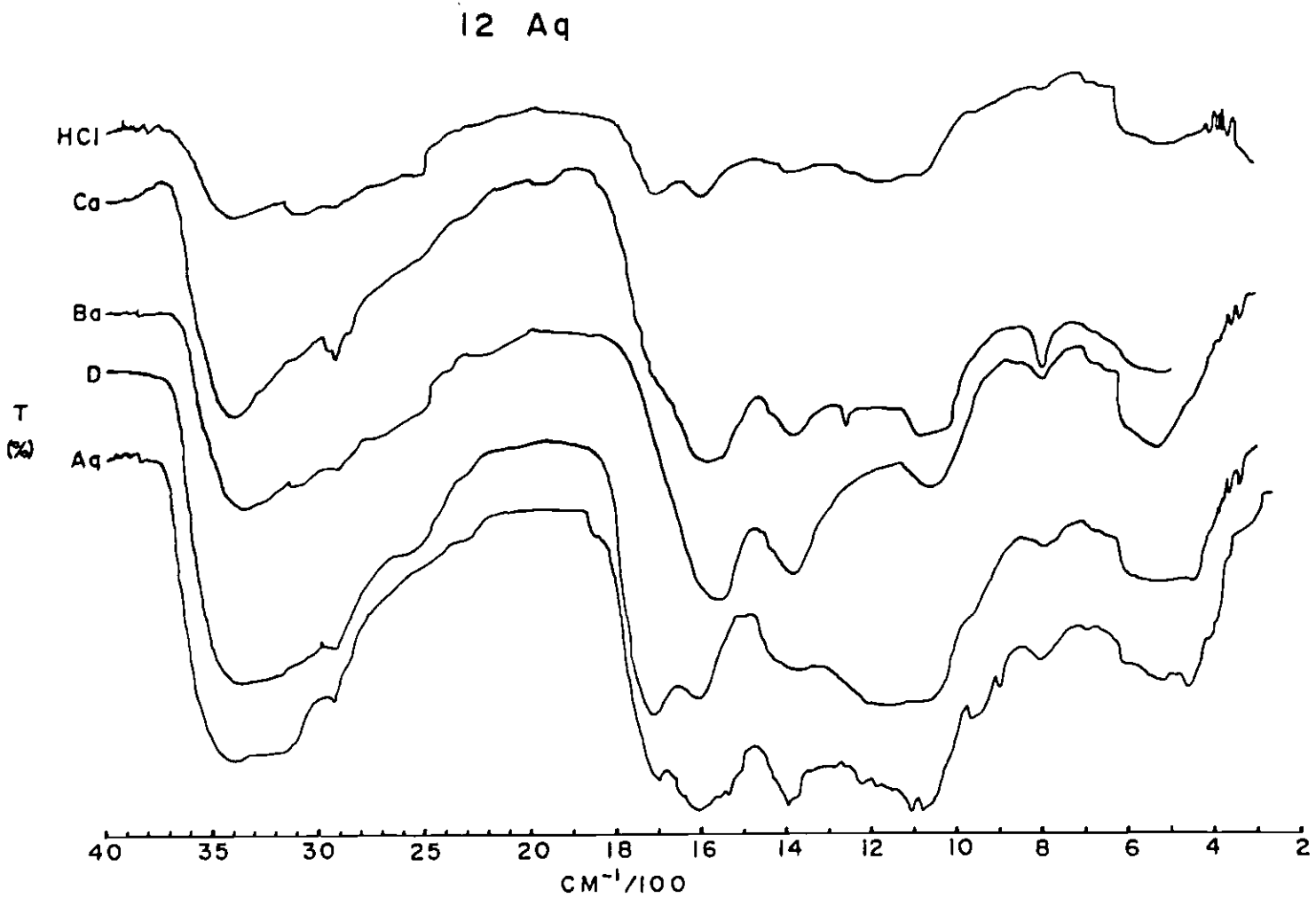


Figure 10. Infrared spectra of aqueous extract of Sample #12 and derivatives.

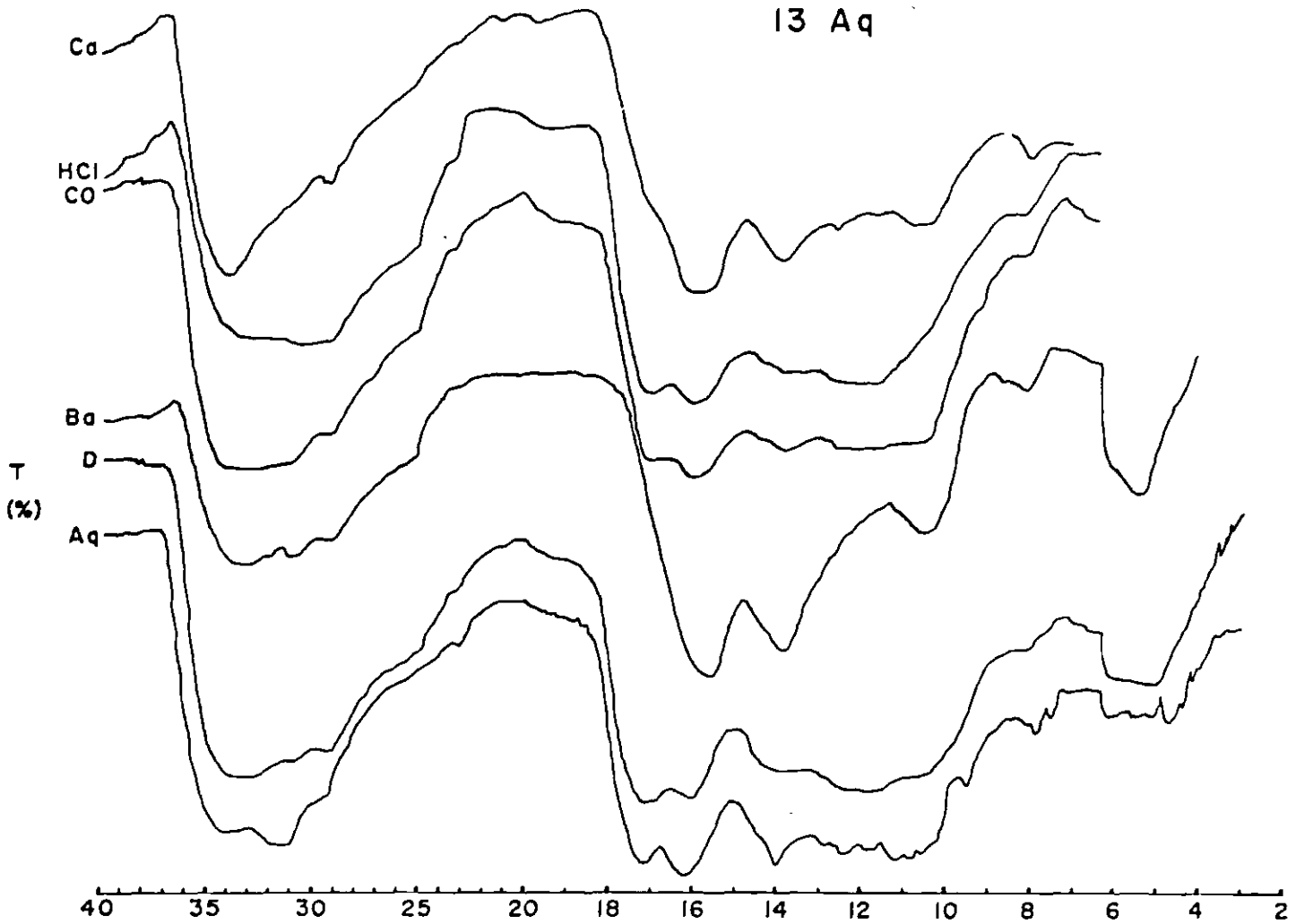


Figure 11. Infrared spectra of aqueous extract of Sample #13 and derivatives.

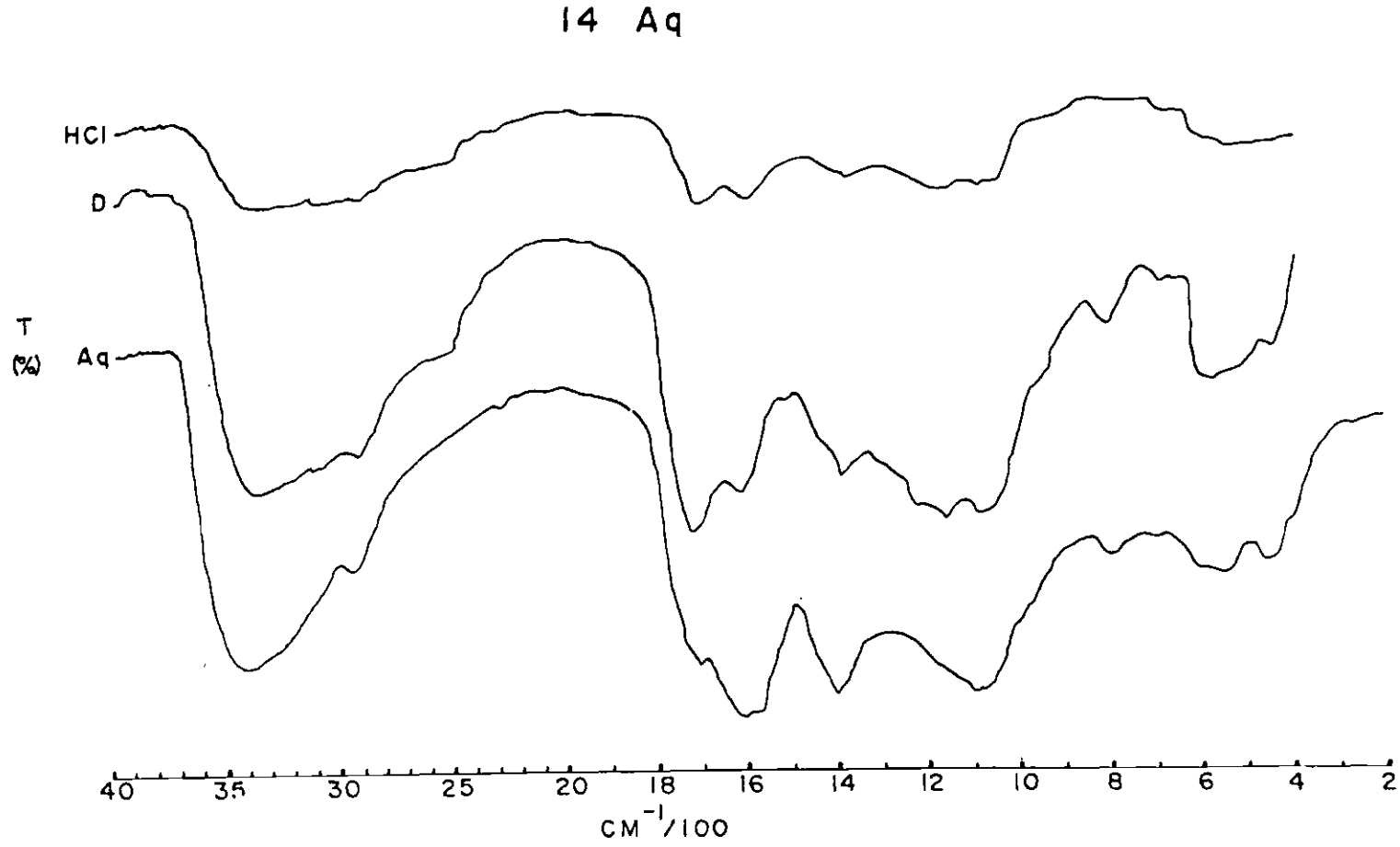


Figure 12. Infrared spectra of aqueous extract of Sample #14 and derivatives.

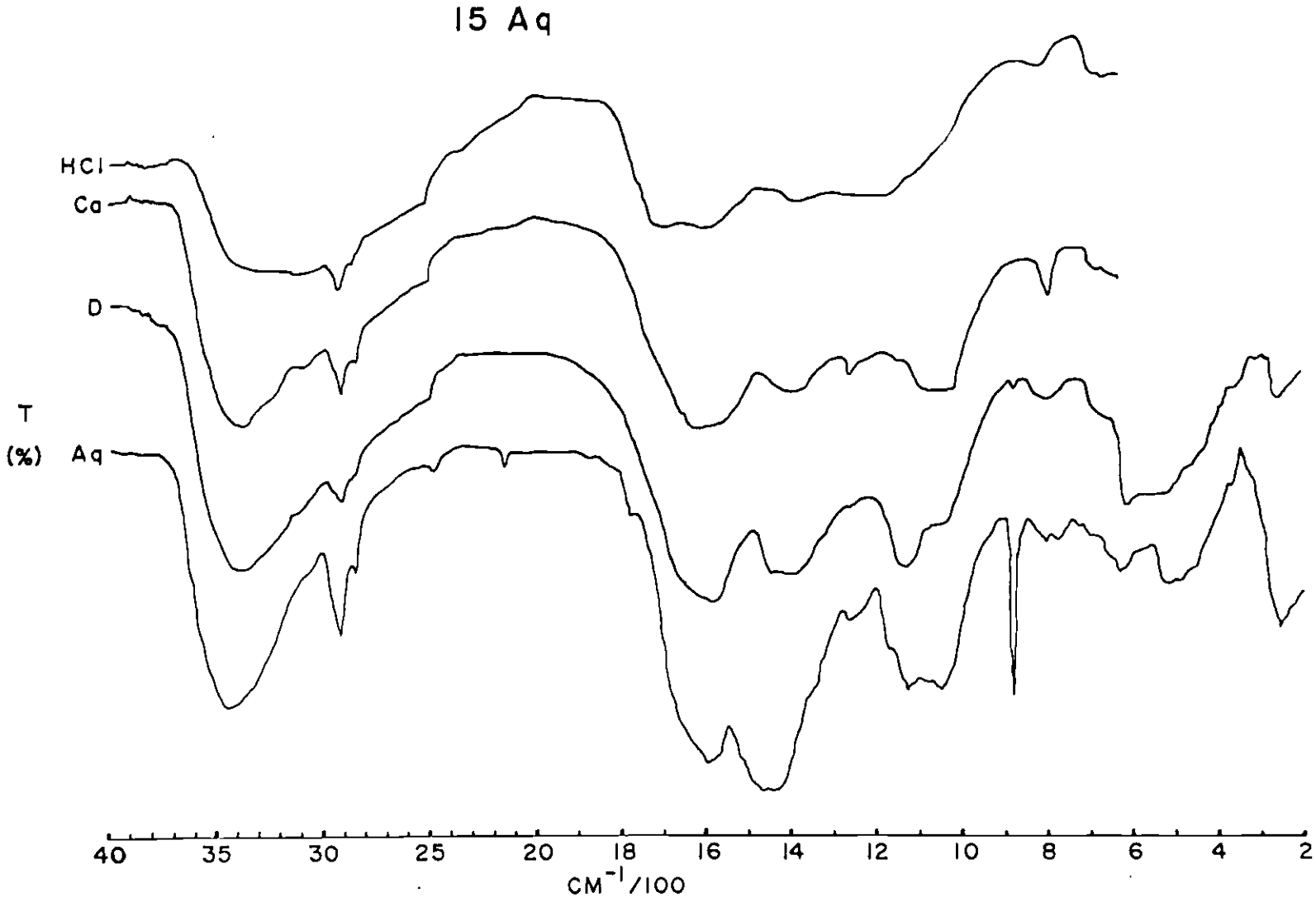


Figure 13. Infrared spectra of aqueous extract of Sample #15 and derivatives.

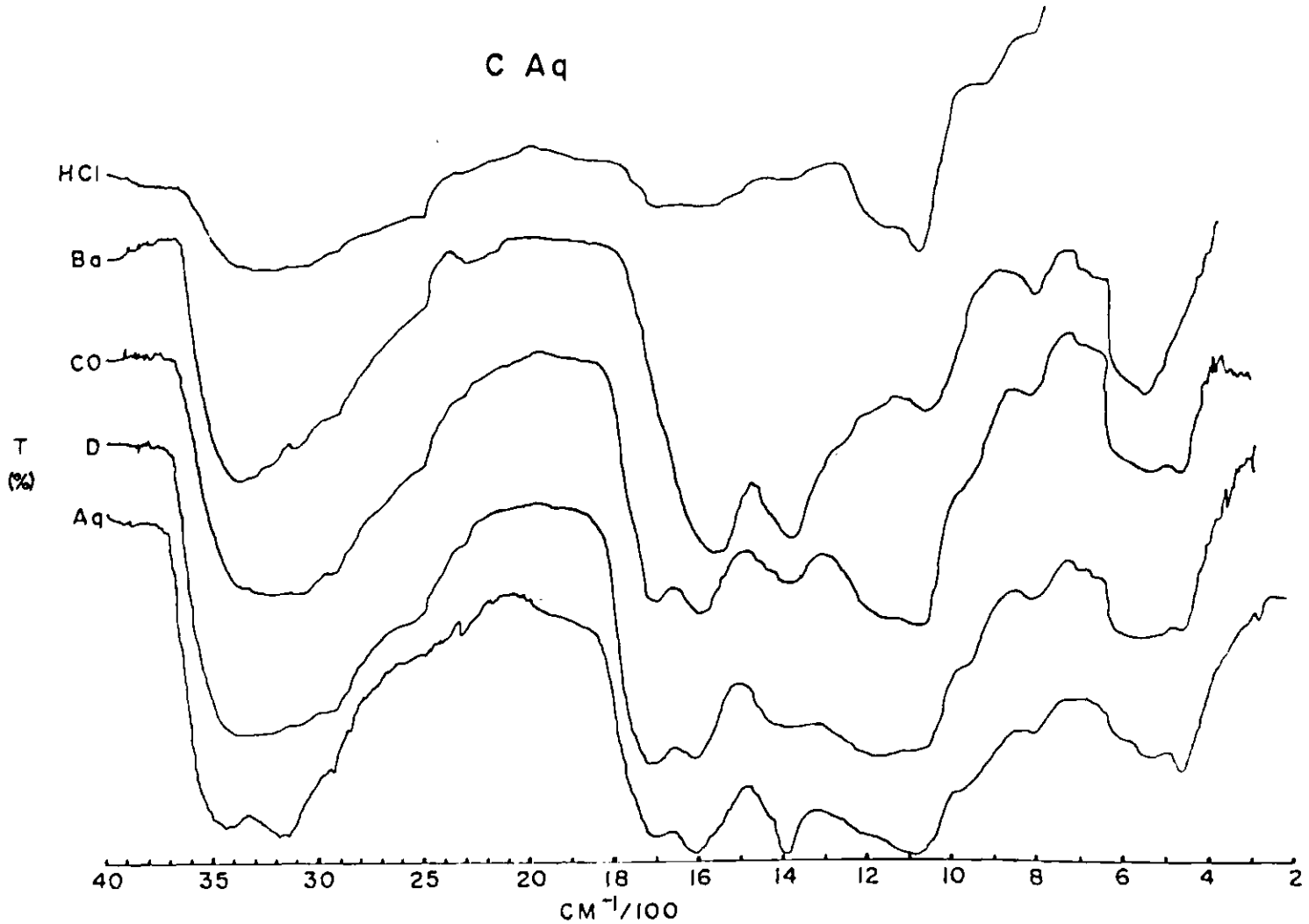


Figure 14. Infrared spectra of aqueous extract of Sample C and derivatives.

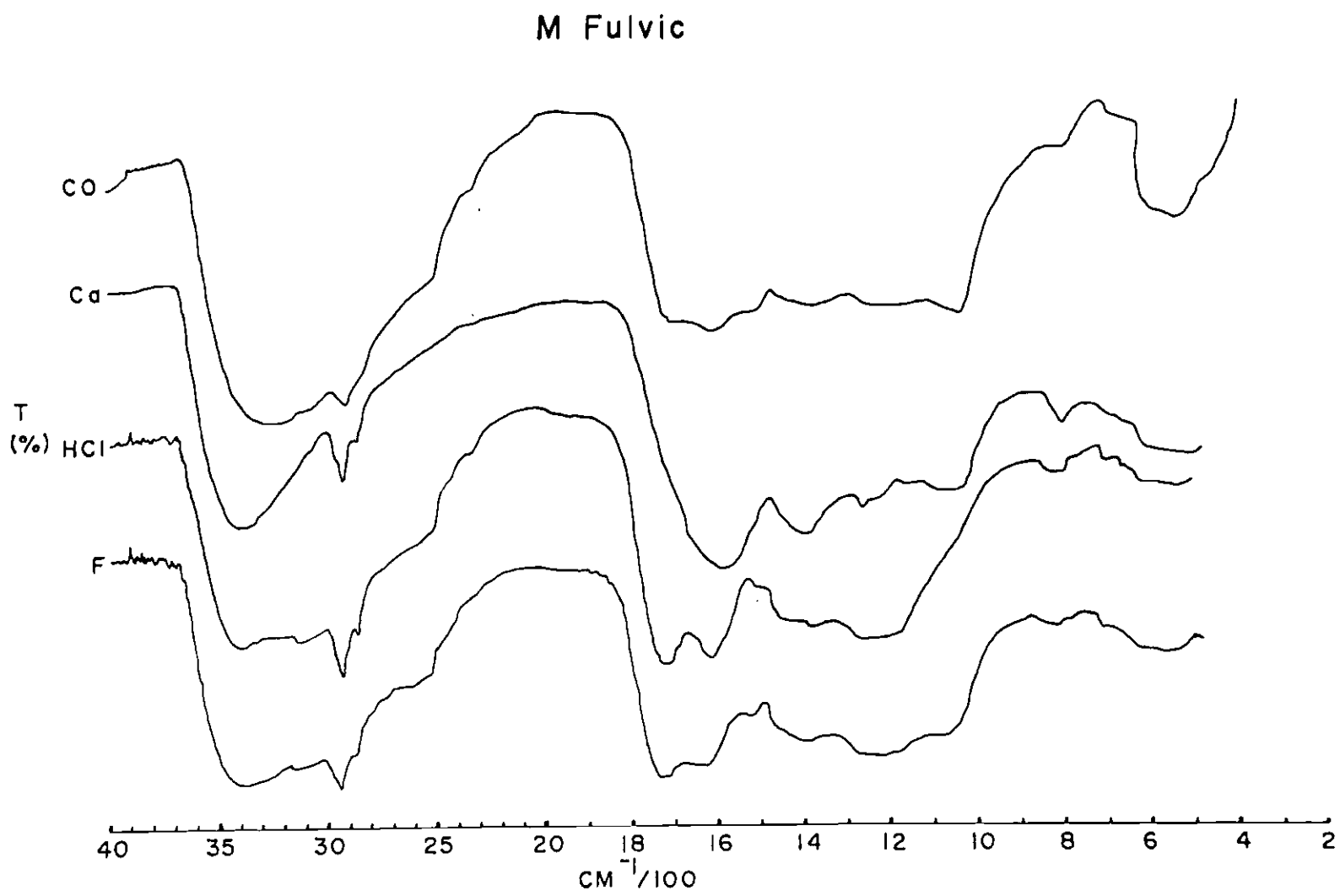


Figure 15. Infrared spectra of M fulvic acid and derivatives.

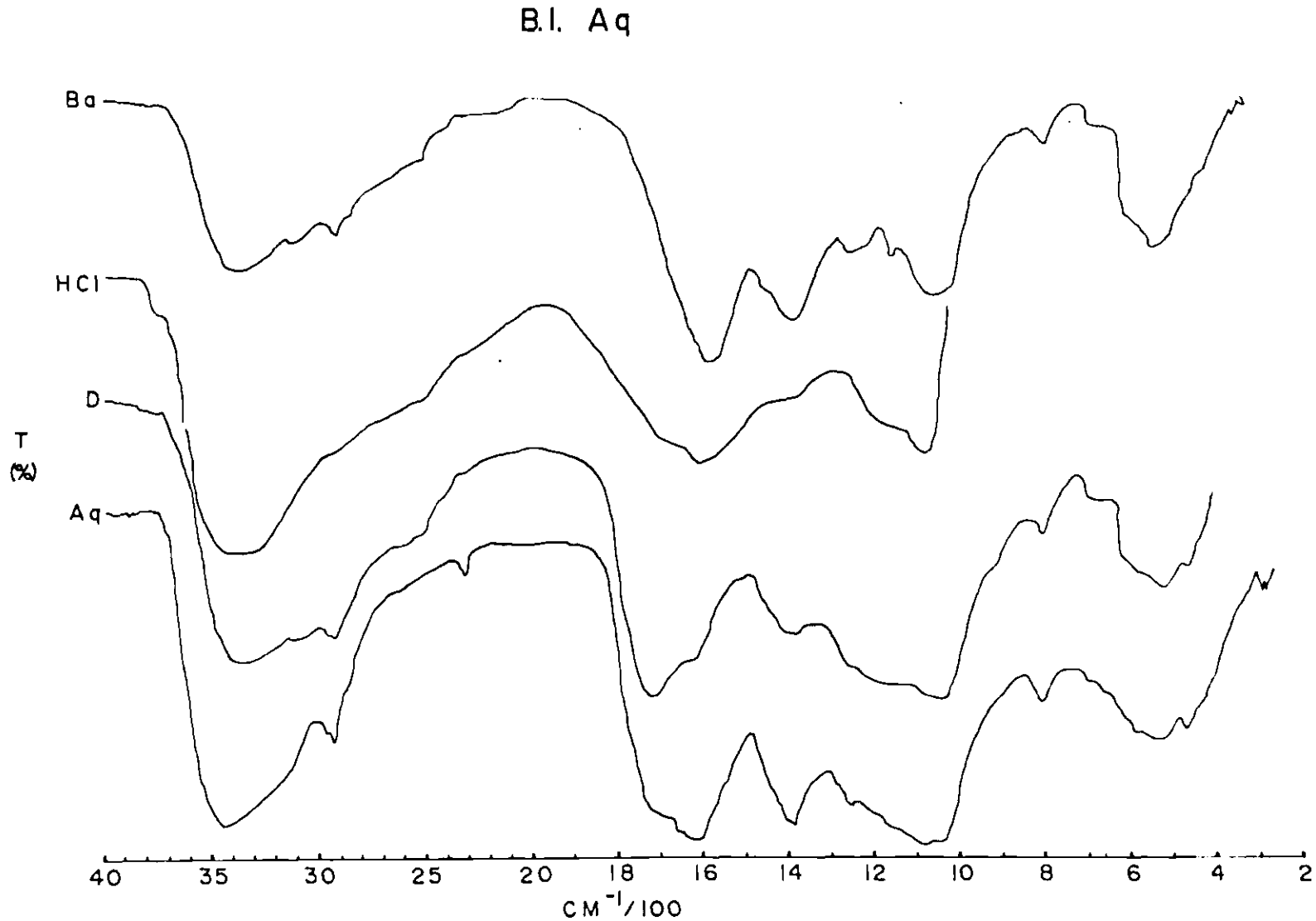


Figure 16. Infrared spectra of aqueous extract of Sample B.I. and derivatives.

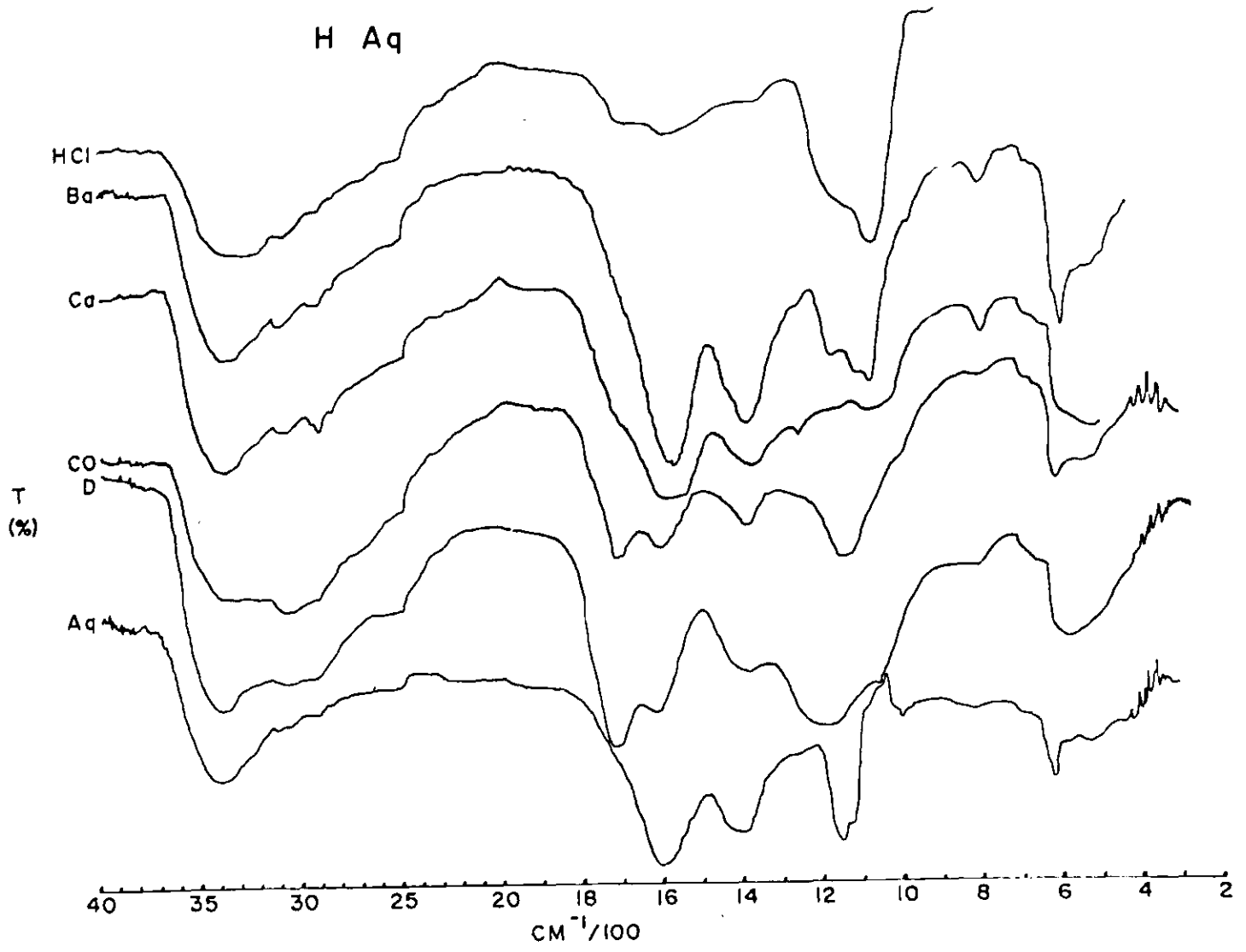


Figure 17. Infrared spectra of aqueous extract of Sample H and derivatives.

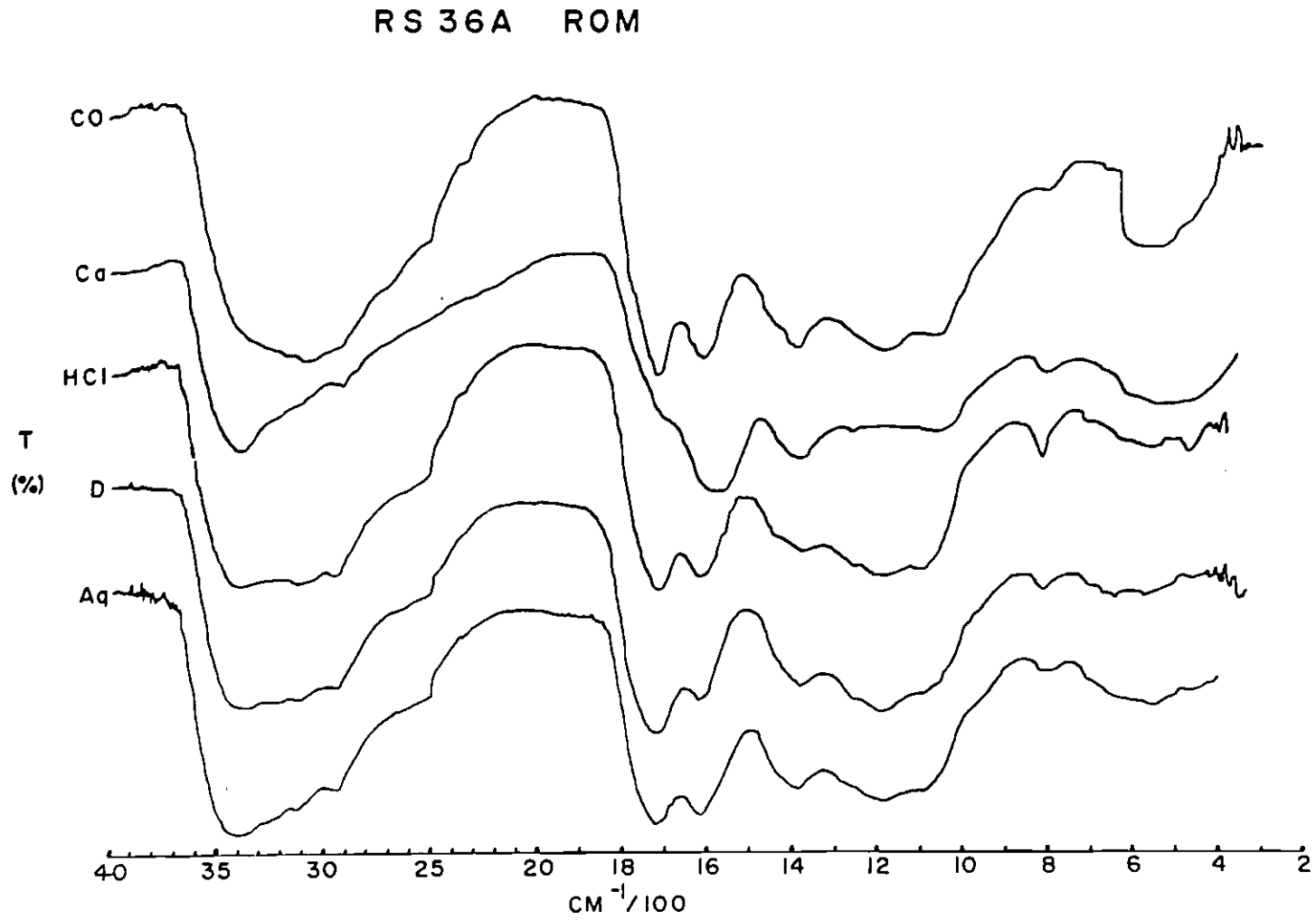


Figure 18. Infrared spectra of river water organic matter Sample RS 36A and derivatives.

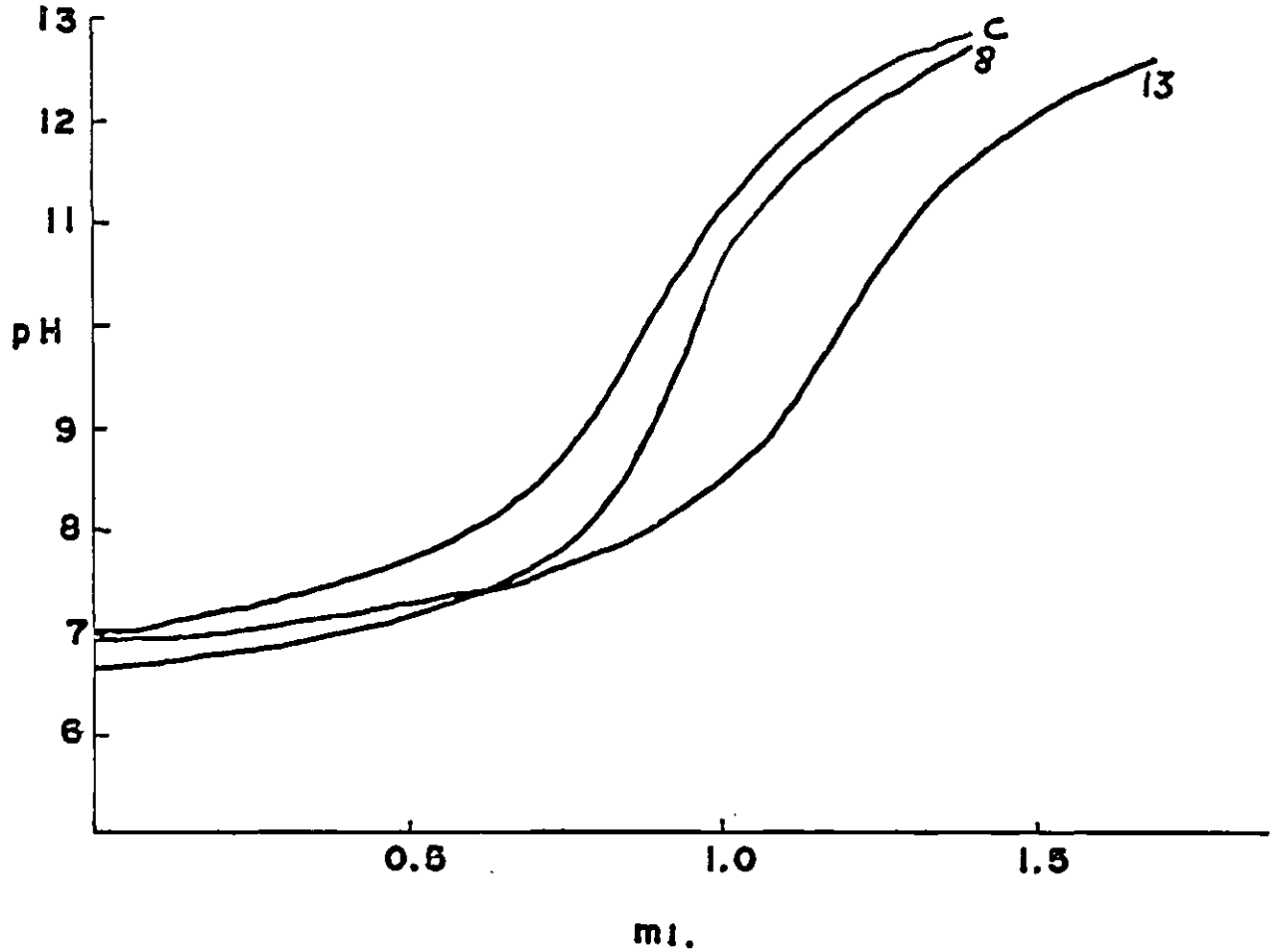


Figure 19. Titration curves of aqueous extracts in pyridine-water solution.

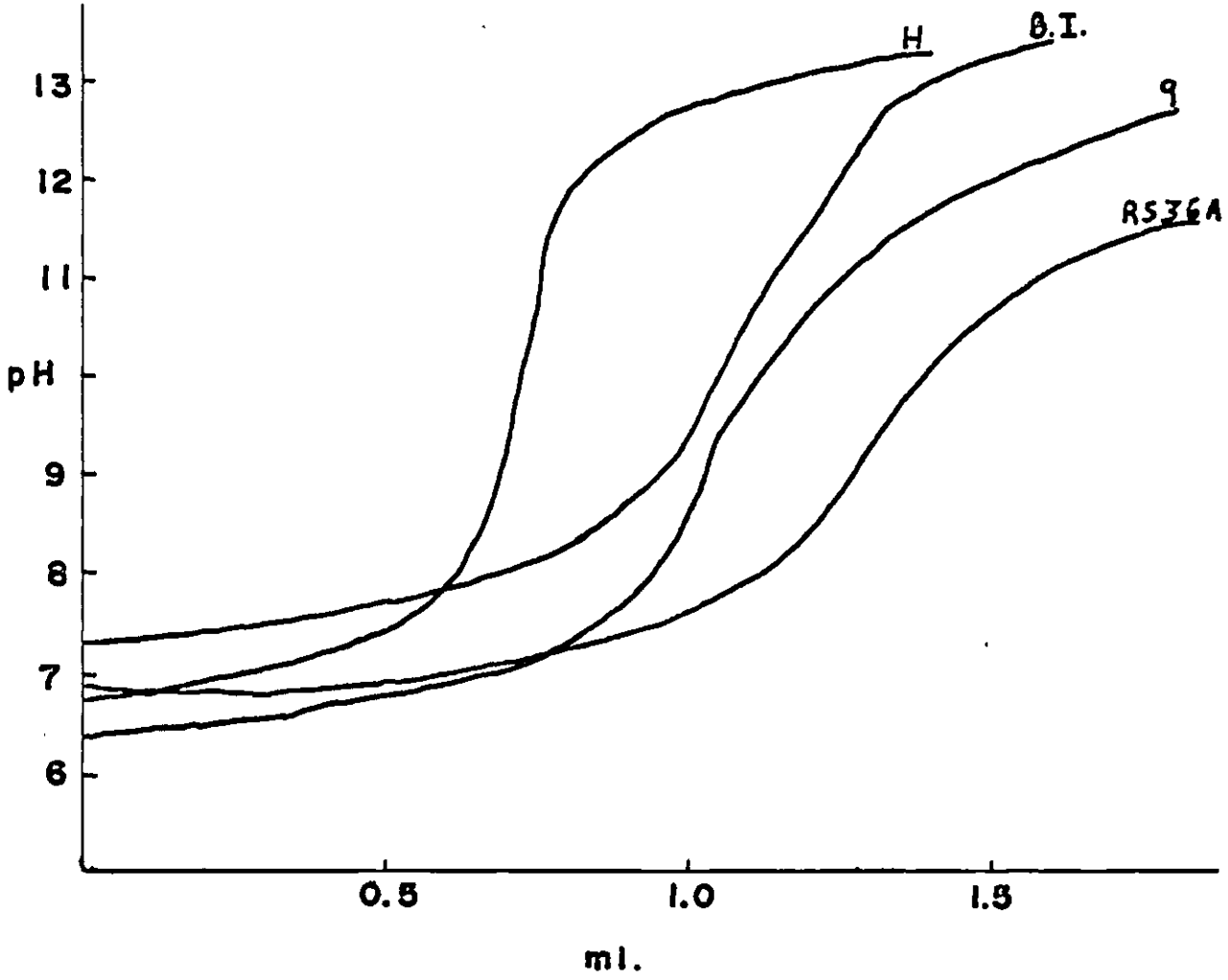


Figure 20. Titration curves of aqueous extracts in pyridine-water solution. (Continued)

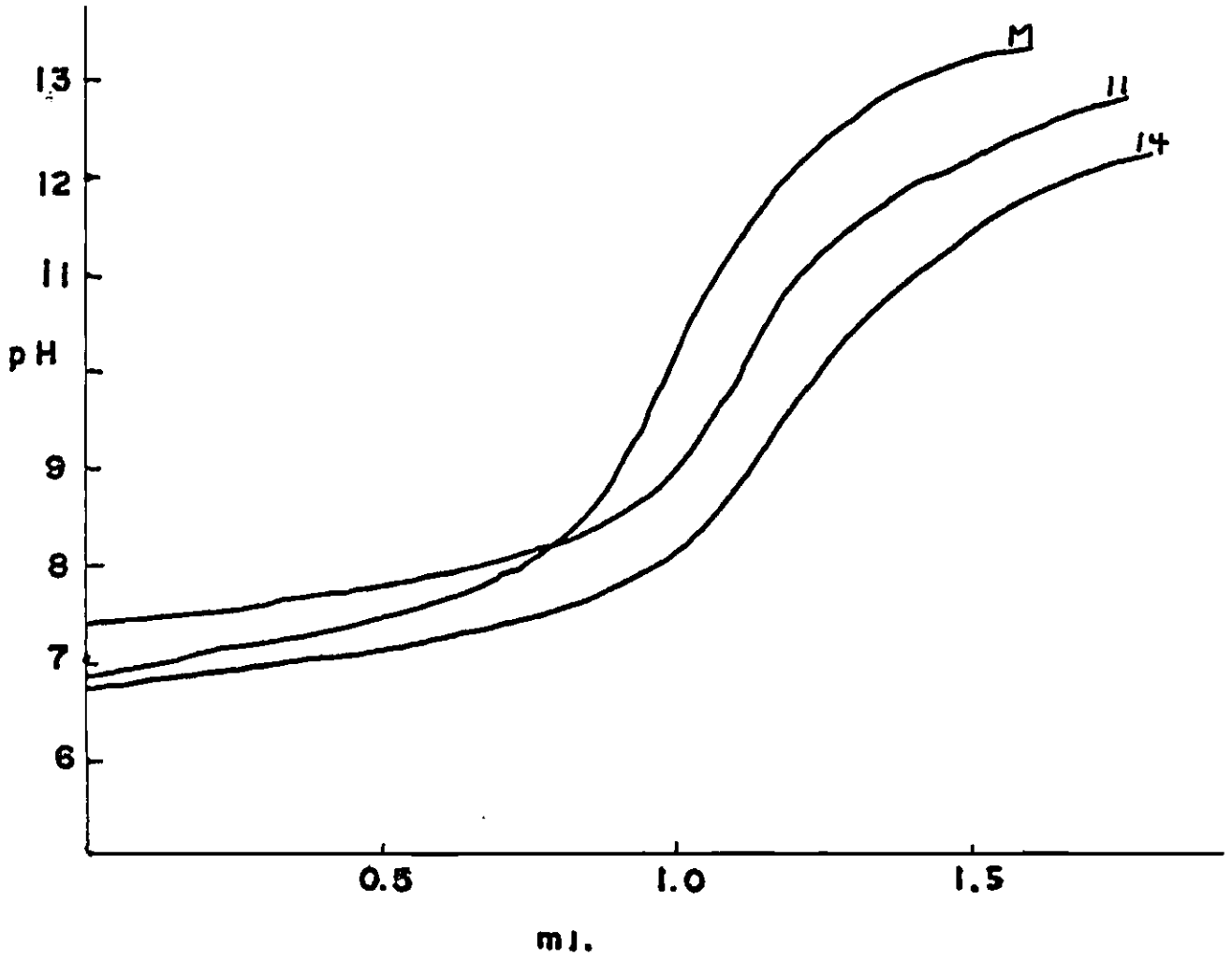


Figure 21. Titration curves of aqueous extracts in pyridine-water solution. (Continued)

# BIBLIOGRAPHY

Aleksandrova, L. N. (1972) Study of the Humification of Plant Residues and of the Nature of Newly Formed Humic Acids. Soviet Soil Science, 429-437.

Anderson, H. A., Hepburn, A., and Sim, A. (1978) Ether-Soluble Hydrolysis Products in Humic and Fulvic Acids. Journal of Soil Sciences, 29, 84-87.

Andren, Anders, W., and Harriss, Robert C. (1975) Observations on the association between mercury and organic matter dissolved in natural waters. Geochim. et Cosmochim.. Acta, 39, 1253-1257.

Beck, K. C., and Reuter, J. H. (1974) Organic and inorganic geochemistry of some coastal plain rivers of the southeastern United States. Geochim. et Cosmochim. Acta, 38, 341-364.

Borggaard, Ole K., (1974) Titrimetric determination of acidity and pK values of humic acid. Acta Chem. Scand. 28, 121-122.

Casagrande, Daniel J., and Given, P. H. (1974) Geochemistry of amino acids in some Florida peat accumulations-I. Analytical approach and total amino acid concentrations. Geochim. et Cosmochim. Acta, 38, 419-434.

Casagrande, Daniel J., (1976) Organic Geochemical Investigations. In Environments of Coal Formation, The Pennsylvania State University.

Christman, R. F., and Chassemi, M. (1966) Chemical nature of organic color in water. Jour. AWWA, 58, 723-741.

Cohen, Arthur D., (1973) Petrology of some holocene peat sediments from the Okeefenokee Swamp-Marsh complex of Southern Georgia. Geological Society of America Bulletin, 84, 3867-3878.

Cohen, Arthur D., (1974) Petrography and paleoecology of holocene peats from the Okeefenokee Swamp-marsh complex of Georgia. Journal of Sedimentary Petrology, 44, 716-726.

Dormaar, J. F., (1967) Infrared spectra of humic acids from soils formed under grass or trees. Geoderma. 1, 37-45.

Dubois, Michel, Billes, K. A., Hamilton, J. K., and Rebers, P. A., (1956) Colorimetric method for determination of Sugars and related substances. Analyical Chemistry, 28, 350-356.

Dunn, Townsend Helme, (1974) Electrode studies of stability constants of Cd-river water organic matter complexes. M. S. Thesis, The Georgia Institute of Technology.

Flaig, W., Beutelspacher, H., and Rietz, E., (1975) Chemical composition and physical properties of humic substances. In Soil Components, 1, Springer-Verlag, N.Y.

Jackson, Togwell A., (1975) Humic matter in Natural waters and sediments. Soil Science, 119, 56-64.

Karpenko, N. P., and Karavayev, N. M., (1966) Freezing method for separating humic acids. Soviet Soil Science, 1154-1156.

Khan, S. U., (1973) Interaction of humic substances with bipyridylium herbicides. Canadian J. Soil Science, 53, 199-204.

Lynch, D. L., Hearns, E. E., and Cotnoir, L. J., Jr., (1957) The determination of polyuronides in soils with carbazole. Soil Science of America Proceedings, 21, 160-162.

Martin, S. J. (1973) Chemical characteristics of dissolved organic matter in river water. M. S. Thesis, The Georgia Institute of Technology.

Orlov, D. S., Rozanova, O. N. and Matyukhna, S. G., (1962) Infrared absorption spectra of humic acids. Soviet Soil Science, 15-21.

Orlov, D. S., (1966) Spectrophotometric analysis of humic substances. Soviet Soil Science, 1278-1286.

Otsuki, Akira, (1967) Some precursors of humic acid in recent lake sediment suggested by infra-red spectra. Geochim. et Cosmochim., 31, 1505-1515.

Packham, R. F., (1964) Studies of organic color in natural water. Proc. Soc. Water Treat. Exam., 13, 316-334.

Reuter, J. H., and Perdue, E. M., (1977) Importance of heavy metal-organic matter interactions in natural waters. Geochim. et Cosmochim. Acta, 41, 325-334.

Riffaldi, R., and Schnitzer, M., (1973) Effects of 6 N HCL hydrolysis on the analytical characteristics and chemical structure of humic acids. Soil Science, 115, 349-356.

Rogers, M. A., (1965) Carbohydrates in aquatic plants and associated sediments from two Minnesota lakes. Geochim. et Cosmochim. Acta, 29, 183-200.

Schnitzer, M., and Griffith, S. M., (1975) Novel method for estimating hydrogen-bonded carboxyl groups in humic substances. Canadian J. Soil Science, 55, 491-493.

Schnitzer, M., and Gupta, Umesh C., (1965) Determination of acidity in soil organic matter. Soil Science Society Proceedings, 274-277.

Schnitzer, M., and Khan, S. U., (1972) Humic substances in the environment. Marcel Dekker, Inc., New York.

Stevenson, F. J., and Cheng, C. N., (1970) Amino acids in sediments: Recovery by acid hydrolysis and quantitative estimation by a colorimetric procedure. Geochim. et Cosmochim. Acta, 34, 77-88.

Stevenson, F. J., and Goh, K. M., (1971) Infrared spectra of humic acids and related substances. Geochim. et Cosmochim. Acta, 35, 471-483.

Stevenson, F. J., and Goh, K. M., (1972) Infrared spectra of humic and fulvic acids and their methylated derivatives: Evidence for nonspecificity of analytical methods for oxygen-containing functional groups. Soil Science, 113, 334-396.

Stevenson, F. J. and Goh, K. M., (1974) Infrared spectra of humic acids: elimination of interference due to hygroscopic moisture and structural changes accompanying heating with KBr. Soil Science, 117, 34-41.

Tan, K. H. and Giddens, J. E., (1972) Molecular weights and spectral characteristics of humic and fulvic acids. Geoderma, 8, 221-229.

Theng, B. K. G., Wake, J. R. H. and Posner, A. M., (1966) The infrared spectrum of humic acid. Soil Science, 102, 70-72.

Vinkler, F., Lakatos, B., and Meisel, J., (1976) Infrared spectroscopic investigations of humic substances and their metal complexes. Geoderma, 15, 231-242.

Yekaterinina, N., and Kukharenko, T. A., (1969) Conjugated and non-conjugated forms of carbonyl groups in humic acids. Soviet Soil Science, 497-501.